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ORGANOTHALLIUM COMPOUNDS¹

ROYAL KILBURN ABBOTT, JR.

From the Department of Chemistry, Iowa State College

The chief emphasis in the preparation of the organothallium compounds described in the following has been upon the introduction of new groups into the molecule. In general, the introduction of water-solubilizing groups has received special attention.

The choice of a solubilizing acid ("X group") which will impart at least 1 per cent solubility in water to a diarylthallium salt of the type R_2TlX is limited by the necessity that the group be nontoxic. This criterion excludes such ions as fluoride, although diphenylthallium fluoride is moderately soluble in water. The reaction in boiling pyridine between a diarylthallium halide and the silver salt of a water-soluble acid was investigated as a ready means of preparing such R_2TlX compounds as diphenylthallium sulfanilate, m. p. 345° with decomposition, 0.06 per cent soluble in water at 25° ; dimethylthallium saccharide, m. p. $231-233^\circ$, more than 1 per cent soluble in water; diethylthallium saccharide, m. p. $220-221^\circ$, 1.55 per cent soluble in water at 25° ; diphenylthallium saccharide, m. p. $315-320^\circ$ with slight decomposition, 0.17 per cent soluble in water at 25° ; and di-2-pyridylthallium lactate, m. p. $205-208^\circ$ with decomposition, more than 1 per cent soluble in water.

Direct nuclear substitution was also studied as a means of introducing functional groups into diarylthallium salts. It was found that di-*o*-tolylthallium bromide could be sulfonated by fuming sulfuric acid at -20° to give di-2-(4-sulfotoluene)thallium sulfate in 50 per cent yield. The compound was somewhat soluble in water, especially on warming. It was readily soluble in 10 per cent sodium hydroxide solution, and the sodium salt could be prepared by exact neutralization and removal of the water under reduced pressure. The position of sulfonation was established by cleavage with bromine and neutralization of the resultant *x*-bromotoluenesulfonic acid with thallous hydroxide. The thallous *x*-bromotoluenesulfonate (m. p. $220-222^\circ$) thus obtained was found to be identical with an authentic specimen of thallous 2-bromotoluene-4-sulfonate, thus demonstrating that the metal is *meta*-orienting.

The direct nitration of diphenylthallium nitrate yielded di-*m*-nitrophenylthallium nitrate, a pale yellow powder, readily soluble in hot pyridine. It underwent gradual decomposition at temperatures above 300° . It was found, however, that this compound could be better prepared from *m*-nitrophenylboric acid and thallium trichloride.

Detailed directions are given for the preparation of thallous hydroxide from thallous sulfate, and for thallous ethoxide from thallous hydroxide. Diethylthallium ethoxide was prepared from diethylthallium chloride

¹ Original thesis submitted December 8, 1942. Doctoral thesis number 695A.

and thallos ethoxide in pyridine. It was also prepared from diethylthallium chloride and the cheaper and more readily prepared sodium ethoxide in absolute alcohol. By either method the yield was approximately 70 per cent of a compound which boiled as a pale yellow liquid at 101-102° under 0.1 mm. pressure, and solidified on cooling to a nearly colorless, crystalline mass, which melted at 43-45°. The compound was immediately soluble in water because of complete hydrolysis, and had an odor somewhat resembling tetraethyllead.

The preparation of several R_2TlX compounds with functional groups was undertaken by halogen-metal interconversion of the appropriate halides with butyllithium. Di-*o*-hydroxyphenylthallium bromide was prepared from *o*-bromophenol and thallium trichloride in 15 per cent yield. It did not melt at 340°. Di-2-pyridylthallium chloride was likewise prepared from 2-bromopyridine in 80 per cent yield. It melted at 288-291° and was completely insoluble in water.

Thallium trichloride-tripyrindine was prepared from ether solutions of thallium trichloride and pyridine. Recrystallized several times from absolute alcohol, it melted at 148-150° without decomposition. It could not be rearranged to an organothallium compound by heating with pyridine in a sealed tube at 180° for 10 hours. Thallium tribromide-tripyrindine was prepared in 75 per cent yield by the oxidation of a suspension of thallos bromide in pyridine with pyridinium bromide. The compound was readily recrystallized from absolute alcohol and then melted at 113-115° without decomposition. Also, from the thallic halide in ether were prepared: in 79 per cent yield, thallium trichloride tri-(2-bromopyridine) melting at 145-146°; in 68 per cent yield, thallium trichloride tri-(2-aminopyridine hydrochloride) melting with decomposition at 121-125°; and in 81 per cent yield, thallium trichloride-cysteine hydrochloride melting at approximately 350° with decomposition. This last compound was 0.29 per cent soluble in water.

An improvement was found on the known procedure for the preparation of *o*-bromodimethylaniline from *o*-bromoaniline and methyl sulfate. The compound was found to boil at 101-102° under 12 mm. pressure. The colorless oily liquid, obtained in 95 per cent yield, was a glass both at 0° and at -75°. The following physical constants were obtained: n_D^{25} 1.5748, d_4^{25} 1.3880. The picrate melted sharply at 150-151°. The picrate of the starting material, *o*-bromoaniline, melted at 127-128°. From the *o*-bromodimethylaniline the Grignard reagent was prepared in 62 per cent yield, and from this Grignard reagent and thallium trichloride in ether solution di-*o*-dimethylaminophenylthallium bromide was obtained in 60 per cent yield.

The reaction between *p*-dimethylaminophenyllithium and thallium trichloride did not yield the expected di-*p*-dimethylaminophenylthallium bromide, but instead a purple dye. *p*-Dimethylaminophenylboric acid was prepared in 62 per cent yield from *p*-dimethylaminophenyllithium and *n*-butyl borate. It melted at 243-245° with decomposition. The structure was proved by analysis and by the reaction with mercuric chloride to

yield the known *p*-dimethylaminophenylmercury chloride, melting at 224-226° with decomposition. *p*-Dimethylaminophenylboric acid likewise reacted with an aqueous solution of thallium trichloride to give a purple dye and not the expected di-*p*-dimethylaminophenylthallium chloride. The desired di-*p*-dimethylaminophenylthallium bromide was finally obtained by the action of *p*-dimethylaminophenyllithium on a suspension of thallous bromide in ether. The yield based on one-third of the total thallium available in the reaction—since two-thirds is necessarily reduced to the free metal—was 68 per cent. The compound did not melt at 350°.

Di-*p*-anisylthallium bromide was prepared in 48 per cent yield from *p*-anisylmagnesium bromide and an ether solution of thallium trichloride. It did not melt at 330°. By the analogous reaction, di-*o*-anisylthallium bromide was prepared in 47 per cent yield. It did not melt at 330°. These two organothallium compounds could not be made to undergo coupling with *p*-nitrobenzenediazonium chloride either in aqueous, ethyl acetate or dilute pyridine suspension. *p*-Nitrobenzenediazonium chloride was found to cleave di-*p*-dimethylaminophenylthallium bromide to yield 4'-nitro-4-dimethylaminoazobenzene. A blank experiment showed that the cleavage was not merely due to the free acid present, which does not ordinarily cleave R₂TlX compounds.

The following thallous salts were prepared in connection with various phases of the work, but primarily because they often possess good melting point characteristics: 2,4,6-trinitrobenzoate, melting at 160-163° with decomposition and gas evolution; oxalate, melting at 315-320° with decomposition and gas evolution; naphthalene-β-sulfonate, melting at 234-236°; benzenesulfonate, melting at 185-187°; laurylsulfonate, melting at 143-145°; *p*-toluenesulfinate, melting at 154-156°; monothallous phenylphosphonate, melting at 200-201°; dithallous phenylphosphonate, melting at 317-320°; diphenylphosphonate, melting at 203-205°; thallous salt of nitromethane, decomposing gradually above 160°; thallous salt of nitroethane, melting at 80-82° with decomposition and gas evolution; methylmercaptide, melting at 136-140° with decomposition; ethylmercaptide, decomposing around 100° without melting; *n*-butylmercaptide, melting at 84-90° with decomposition; thiophenolate, melting at 258-260°; *p*-thiocresolate, melting at 178-180°; thio-β-naphtholate, melting at 165-168°; and terephthalate, which did not melt at 340°.

ORGANOTIN COMPOUNDS¹

CLYDE EDWARD ARNTZEN

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A review of the literature on organotin compounds is given, including tables containing the known organotin compounds with their melting or boiling points and references to the preparations and attempted preparations of each compound. Complexes of organotin compounds with other organotin compounds, ammonia, and amines are also included. The preparations, physical properties, and reactions of organotin compounds are discussed in general. Some correlations are made between the methods of preparation, physical properties, and reactions of organotin compounds with those of the other organometallic compounds of the B-family of Group IV. The nomenclature of several organotin compounds is modified to conform with the present tendency of using the ending *tin* instead of *stannane* for these compounds. Included in this review is also a survey of the therapeutic applications of organotin compounds.

Prior to the preparation of organotin compounds several halogen-metal interconversion reactions were investigated. Several of the halogen-metal interconversion products were useful in the preparation of organotin compounds. The halogen-metal interconversion reactions between *n*-butyllithium and halogen-substituted phenols, after carbonation with solid carbon dioxide and acidification, gave the corresponding hydroxybenzoic acids. From the reactions of *o*-bromophenol, *p*-bromophenol, and *p*-iodophenol with *n*-butyllithium, there was obtained, after carbonation and acidification, respectively, *o*-hydroxybenzoic acid (67 per cent), *p*-hydroxybenzoic acid (35 per cent), and *p*-hydroxybenzoic acid (50 per cent).

Low temperatures and short-time reactions made possible halogen-metal interconversions between *n*-butyllithium and halogen-substituted benzoic acids. There was obtained, after carbonation and acidification, 35, 12, and 62 per cent yields of the corresponding carboxybenzoic acids from *n*-butyllithium and *o*-bromo-, *o*-iodo-, and *p*-iodobenzoic acids, respectively. The experimental evidence indicated that the halogen-metal interconversion reaction was very rapid.

Not only could halogen-metal interconversions be effected with halogen-substituted benzoic acids but also with halogen-substituted *N*, *N*-dialkyl sulfonamides. In order to study the interconversion reaction with this type of compound the new compound, *p*-iodo-*N,N*-diethylbenzenesulfonamide (m.p. 57-58.5°), was prepared from *p*-iodobenzenesulfonyl chloride and diethylamine. At -- 75°, *p*-iodo-*N,N*-diethylbenzenesulfonamide and *n*-butyllithium, after carbonation and acidification, gave a

¹ Original thesis submitted December 7, 1942. Doctoral thesis number 693A.

72 per cent yield of the new compound, *p*-carboxy-*N,N*-diethylbenzenesulfonamide (m.p. 192-194° with turbidity).

The preparation of tetraethyltin as described by Harada² from ethyl bromide and an alloy of sodium, zinc, and tin was investigated and it was shown that more than a 60 per cent excess of ethyl bromide is necessary for a smooth reaction and good yield of tetraethyltin. Harada merely states that a large excess of ethyl bromide was employed.

By employing a procedure analogous to that used for the preparation of tetraphenyllead and by modifying the previously described directions for the preparation of tetraphenyltin, the large scale laboratory production of tetraphenyltin in a minimum amount of time and in a yield of 92 per cent was made possible. The phenylmagnesium bromide was prepared by cooling the reaction vessel in an ice bath. The solvent used for the reaction between phenylmagnesium bromide and stannic chloride was a mixture of ether and toluene.

The application of halogen-metal interconversion products for the preparation of organotin compounds containing functional groups was demonstrated by the preparation of several of these compounds. In the preparation of R_3SnR' and $R_2SnR'_2$ compounds containing hydroxy groups disproportionation or metal-metal interconversion reactions took place with the formation of R_4Sn compounds, unless the organolithium compound was converted to the Grignard reagent by means of magnesium bromide prior to the reaction with R_3SnX or R_2SnX_2 compounds. Although organolead compounds also yield some R_4Sn compound in similar reactions, this tendency was shown to be more pronounced in the organotin series.

Triethyl-*o*-hydroxyphenyltin was prepared, in 54 per cent yield, from the halogen-metal interconversion product of *o*-bromophenol and *n*-butyllithium by conversion to the Grignard reagent and reaction with triethyltin bromide. The boiling point of triethyl-*o*-hydroxyphenyltin was 155-156° at 15 mm. The density and index of refraction were: d_4^{25} 1.3150 and n_D^{25} 1.5379. Kocheshkov and co-workers³ prepared triethyl-*o*-hydroxyphenyltin from hexaethylditin and di-*o*-hydroxyphenylmercury. These authors report the boiling point as 197-200° at 3 mm. The density and index of refraction of triethyl-*o*-hydroxyphenyltin prepared from the halogen-metal interconversion product, however, compare favorably with those reported by Kocheshkov and co-workers.

In an analogous manner the following new organotin compounds were prepared from halogen-metal interconversion products, after conversion to the Grignard reagent prior to reaction with the organotin halides: triphenyl-*o*-hydroxyphenyltin (m.p. 176-177° with decomposition and depending on the rate of heating) in a yield of 57 per cent from *o*-bromophenol, *n*-butyllithium, magnesium bromide, and triphenyltin chloride; triphenyl-*p*-hydroxyphenyltin (m.p. 201-203° depending on the rate of heating) in a yield of 10 per cent from *p*-bromophenol, *n*-butyllithium,

² Harada, *Sci. Papers Inst. Phys. Chem. Research* (Tokyo), 35, 290 (1939).

³ Kocheshkov, Nesmeyanov, and Puzyreva, *Ber.*, 69, 1639 (1936).

magnesium bromide, and triphenyltin chloride; di-*o*-hydroxyphenyldiphenyltin (m.p. 136-138° depending on the rate of heating) in a yield of 68 per cent from *o*-bromophenol, *n*-butyllithium, magnesium bromide, and diphenyltin dichloride; triphenyl-*o*-hydroxymethylphenyltin (m.p. 158-159°) in a yield of 64 per cent from *o*-bromobenzyl alcohol, *n*-butyllithium, magnesium bromide, and triphenyltin chloride; triphenyl-*p*-hydroxymethylphenyltin (m.p. 98-100°) in a yield of 66 per cent from *p*-bromobenzyl alcohol, *n*-butyllithium, magnesium bromide, and triphenyltin chloride; triphenyl-*o*-methoxymethylphenyltin (m.p. 94.5-95.5°) in a yield of 35 per cent from *o*-bromobenzylmethyl ether, magnesium bromide, *n*-butyllithium, and triphenyltin chloride, also in a yield of 38 per cent from the Grignard reagent prepared directly from *o*-bromobenzylmethyl ether and magnesium.

Two new organotin compounds containing the dimethylaminophenyl groups were prepared: triphenyl-*o*-dimethylaminophenyltin (m.p. 110-112°) in a yield of 64 per cent from *o*-dimethylaminophenylmagnesium bromide and triphenyltin iodide, and also from *o*-dimethylaminophenyllithium and triphenyltin iodide; triphenyl-*p*-dimethylaminophenyltin (m.p. 132-134°) in a yield of 62 per cent from *p*-dimethylaminophenyllithium and triphenyltin chloride.

Several attempts were made to prepare triphenyl-*p*-aminophenyltin from the reaction of the halogen-metal interconversion product of *n*-butyllithium and *p*-bromoaniline with triphenyltin iodide. When the halogen-metal interconversion product was used directly no pure products could be isolated from the reaction mixture. When the halogen-metal interconversion product was converted to the Grignard reagent by means of magnesium bromide, a small amount of a product melting at 167-169°, separated as the hydrochloride, was obtained. Although this product probably was impure triphenyl-*p*-aminophenyltin, the tin analyses were too high to be conclusive. Separation of this product through the hydrochloride was unsatisfactory since cleavage occurred simultaneously with the formation of the hydrochloride.

Triphenyl-*p*-hydroxymethylphenyltin was oxidized to the new compound triphenyl-*p*-carboxyphenyltin (m.p. 166-168°), in a yield of 44 per cent, with potassium permanganate in alcohol-free acetone. Chambers and Scherer¹ attempted to prepare this compound from triphenyltin-sodium and sodium *p*-bromobenzoate in liquid ammonia but were able to isolate only triphenyltin hydroxide and benzoic acid from the reaction mixture.

The coupling reaction between organotin compounds containing functional groups which facilitate azo compound formation with diazonium chlorides leads also to the formation of organotin cleavage products. Of the several coupling reactions attempted, only in the case of triphenyl-*p*-dimethylaminophenyltin could a pure tin-containing azo compound be isolated, and then only after repeated crystallization. The new compound, triphenyl-4-dimethylamino-3-(4'-nitrophenylazo)-phenyltin (m.p. 190-

¹ Chambers and Scherer, *Jour. Am. Chem. Soc.*, 48, 1054 (1926).

192°), was obtained from triphenyl-*p*-dimethylaminophenyltin and *p*-nitrobenzenediazonium chloride in a mixture of ethyl acetate and aqueous sodium acetate.

Attempts to introduce the sulfonyl chloride group directly into tetraphenyltin led only to cleavage products even at -75° with no evidence of chlorosulfonation. When excess chlorosulfonic acid was used and the mixture then treated with ammonium hydroxide, there was isolated some benzenesulfonamide in addition to metastannic acid. When two equivalents of chlorosulfonic acid were used, some unreacted tetraphenyltin and diphenyl sulfone in addition to cleavage products were isolated.

Although organothallium and organomercury compounds have been prepared from arylboric acids and the corresponding inorganic halides, organotin compounds could not be obtained by this procedure from phenylboric acid and stannic chloride. Most of the phenylboric acid was recovered from these attempted reactions, but apparently some was hydrolyzed to benzene and boric acid by the prolonged treatment with the hot aqueous solution. However, it is possible that a small amount of organotin compound may have been formed but was hydrolyzed under the conditions of the experiment. It was hoped that this reaction would provide a method for the direct preparation of organotin compounds containing functional groups, but since the reaction was unsuccessful in the case of phenylboric acid, the reaction with substituted arylboric acids was not investigated.

The synthesis of an organotin compound containing an aldehyde group has not been accomplished. Many of the usual reactions for the preparation of aldehydes cannot be used since the conditions employed would break the carbon-tin linkage. The Reimer-Tiemann reaction appeared to offer a solution to this problem. Since it was found that tetraphenyltin was not affected by prolonged heating with an alkaline solution such as is required in the Reimer-Tiemann reaction, an analogous experiment was carried out with triphenyl-*o*-hydroxyphenyltin. Unfortunately, triphenyl-*o*-hydroxyphenyltin was cleaved by the alkaline solution, thereby excluding the Reimer-Tiemann reaction as a possible method for the introduction of an aldehyde group into organotin compounds.

THE NATURE OF THE STARCH-IODINE COMPLEX¹

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Evidence now exists that starch is a heterogeneous mixture of a straight-chain glucose polymer called amylose and a branched-chain glucose polymer called amylopectin. The introduction of this idea of branching in the starch molecule, as opposed to the tendency to consider starch as a linear molecule similar to cellulose, as well as the idea of a helical and an extended-chain configuration of starch, makes it desirable to reinvestigate starch materials in the light of these concepts. This investigation offers information gathered in a survey of the iodine complexes of various polysaccharide materials chosen to test the more recent theories of starch structure.

There is a definite relationship between the starch-iodine complex and the structure and configuration of the starch used in forming the complex. The absorption spectrum of starch-iodine was found to be similar to the absorption spectrum of the iodine complex of cyclohexaamylose whose structure has been established as a six-membered cyclic glucose polymer which encloses an iodine molecule. This comparison was made by taking the absorption spectrum of a well-formed crystal of cyclohexaamylose-iodine with a microspectograph and an absorption spectrum of a solution of starch-iodine with a spectrophotometer. Both spectra show a broad absorption band with a maximum at approximately 600 millimicrons.

Starch-iodine solutions show a definite dichroism of flow when placed in a concentric cylinder apparatus in which the inner cylinder can be rotated. The observed dichroism is such that the iodine molecules involved in the complex must have their long axes parallel to the length of the starch molecule. Optical studies of crystalline amylose-iodine complex make any but a helical configuration of amylose unfeasible. X-ray patterns of the amylose iodine complex are in accord with a helical configuration with six glucose residues per helix turn.

The absorption curves of iodine in nonpolar solvents and in starch were compared on the basis of molecular extinction coefficients. The comparison discredits the hypothesis that the blue color of the starch-iodine complex is due to iodine merely dissolved in the hydrocarbon lining of starch in a helical configuration. A more tenable explanation of the increased absorbing power of iodine in starch is based upon the orienting influence of the starch helix on iodine molecules and the interaction of the iodine molecules aligned in the helix.

A change in iodine concentration does not change the absorption maximum of the amylose-iodine complex. Iodine is taken up more readily

¹ Original thesis submitted June 1, 1943. Doctoral thesis number 720.

by amylose than by amylopectin, and the absorption is much greater in the case of amylose-iodine. The spectrophotometric titration of amylose with iodine shows an end point when the ratio of glucose residues to iodine molecules is six to one.

The amylose-iodine complex can be formed in the absence of iodide ions. In their presence, amylose takes up iodide ions in addition to iodine, with a limiting value of one iodide ion to two iodine atoms. The presence of other ions affects the iodine complex formation. This effect is attributed to changes in the configuration of the starch itself, as evidenced by changes in precipitability of starch in the presence of electrolytes.

There is a direct relationship between the chain length of starch and the absorption maximum of the corresponding iodine complex. With an increase in chain length, the absorption maximum shifts to longer wave lengths. This was shown by the absorption spectra of amylose samples whose molecular weights had been determined by chemical and physical means. Further evidence for the shift of the absorption maximum with increasing chain length was shown in a study of the absorption spectra of the iodine complex of synthetic polysaccharides. The synthesis of starch from glucose-1-phosphate by the enzyme phosphorylase could be stopped at various stages, the product isolated, and absorption curves taken of the iodine complex. The absorption curves of four such isolated fractions of synthetic starch showed a shift in the absorption maximum from 525 millimicrons to 590 millimicrons. The width of the absorption band and the small degree of the shift make a quantitative determination of chain length on this basis impractical. There is also a direct relationship between the molecular extinction coefficient of the amylose-iodine complex and the molecular size of the amylose, if the coefficient is calculated for solutions in which the amylose is in excess, in which case the absorption is a function of iodine concentration; and the molecular extinction coefficient increases with an increase in amylose chain length.

A relationship exists between the degree of branching in starch and the absorption maximum of the corresponding iodine complex. There is a shift to shorter wave lengths with an increased amount of branching. The color of a starch-iodine complex is not a function of molecular weight, but a function of the length of straight chains or the length of the longest branches present in the starch molecule.

From the difference in light absorption qualities of amylose-iodine and amylopectin-iodine, it is possible to estimate quantities of these materials present in starches. The values thus obtained—29 per cent amylose in lily bulb starch, 20 per cent amylose in potato starch, 18 per cent amylose in corn starch, and 16 per cent amylose in tapioca starch—are in the right order of magnitude but slightly lower than those obtained by other means.

The enzyme phosphorylase prepared from yeast is capable of cleaving (and presumably synthesizing) a 1:6-glucosidic linkage—the linkage involved in branching in the starch molecule—as well as a 1:4-glucosidic

linkage. This was demonstrated by the action of yeast phosphorylase on the limit dextrin from waxy maize starch which has an abundance of 1:6-glucosidic linkages and on amylose which has only 1:4-glucosidic linkages. This evidence explains the synthesis of a high molecular weight but brown-staining polysaccharide with yeast phosphorylase. The product has a large number of short branches similar to glycogen. Potato phosphorylase as ordinarily prepared is capable of cleaving and synthesizing only a 1:4-glucosidic linkage. There is evidence that an enzyme capable of forming a 1:6-glucosidic linkage is present in potatoes but is lost in the process of purification.

PHOSPHATE FIXATION BY KAOLINITIC AND OTHER CLAYS¹

CHARLES A. BLACK

From the Department of Agronomy, Iowa State College

Because of the recent interest in the combination of phosphate with kaolinite by an anion exchange process, a study was made of the conditions under which this phosphate fixation takes place. An attempt was made to determine the importance of fixation by kaolinite relative to that by the free oxides. The kaolinitic materials used were kaolinite from two different sources, the clay fraction of the Cecil clay soil, and hydrated halloysite. Montmorillonite (bentonite) and illite were included for comparison.

Preliminary work suggested the possibility that, in addition to replacing surface-exposed hydroxyl groups, phosphate ions could penetrate into the crystal between the lattice layers and replace some of the hydroxyl ions at the cleavage planes inside the clay particle. If such were the case, the fixation should slowly increase with time, and no definite "saturation value" would be reached until all the hydroxyl groups had been replaced.

Experiments showed that the phosphate fixation increased greatly as the time of contact was lengthened. For example, the Cecil clay (free iron oxides removed) fixed 393 p.p.m. P during a 10-minute period and 42,500 p.p.m. P during a 2-month period from a solution of the same concentration.

Extraction data showed a slow and gradual removal of fixed phosphate with saturated $(\text{NH}_4)_2\text{C}_2\text{O}_4$ adjusted to pH 7 as contrasted to a rapid removal with 0.1 N NaOH. These results indicate again that some of the phosphate was held inside the crystal since the activity of the oxalate ion should be confined largely to the surface of the particles, whereas the action of the NaOH could take place inside as well as outside because of the relatively small size of the hydroxyl ion. The gradual extraction with the oxalate suggests that as the phosphate was removed from outside positions there was a readjustment whereby the phosphate held inside the crystals moved out to where it could be replaced.

Fixation by iron in the above experiments was believed to be of minor importance because of the thorough previous treatment of the clays to remove free iron oxides. Considerable amounts of aluminum were dissolved as a result of shaking the clays with a solution having the same acidity as the phosphate solutions, but many times more aluminum would have been required to account for the large amounts of phosphate fixed. As a further indication that the phosphate held by the clay was not retained as ordinary aluminum phosphate, the behavior of the fixed phosphate towards extraction by various reagents was quite different from that of C.P. aluminum phosphate.

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To obtain a more complete picture of the behavior of the clays, a study was made of the effect of pH, concentration of phosphate in solution, and time of contact on the amount of phosphate retained in equilibrium systems. The reaction range employed was approximately pH 3 to pH 7.5, the phosphate concentrations were 1, 10, and 100 p.p.m. P in solution, and the times of contact were 48 hours and 30 days.

The shape of the pH-fixation curves for the kaolinitic clays showed that fixation by hydroxyl replacement was very strongly influenced by the phosphate concentration and the time of contact. More phosphate was fixed by this mechanism from the more concentrated solutions and during the longer fixation period. The more finely divided samples showed fixation by hydroxyl replacement at a lower phosphate concentration and at a shorter time of contact than did the coarser clays. Kaolinite which had been ground in a ball mill was the only sample showing this fixation from a solution having an original concentration of 1 p.p.m. P. The coarser sample of kaolinite employed (2 per cent finer than 0.2 micron in diameter) did not show definite fixation due to hydroxyl replacement under any of the conditions employed. The anomalous behavior of hydrated halloysite which, even though it was not finely divided, fixed large amounts of phosphate due to hydroxyl replacement from a solution concentration of 100 p.p.m. P can probably be explained on the basis of easier penetration of phosphate between the lattice layers resulting from structural differences in the clay.

The only type of fixation definitely evident from the pH-fixation curves for finely ground kaolinite was that due to hydroxyl replacement. The curves for the other clays showed modifications resulting from other fixation mechanisms. The apparent effect of aluminum was noted especially in the curves for fixation from solution concentrations of 1 and 10 p.p.m. P, in the form of a maximum at pH 5-7. At these phosphate concentrations, retention by aluminum was more marked after the 30-day fixation period than after the 48-hour interval. In the 100 p.p.m. P solutions, the effect of aluminum was usually masked by the much greater fixation due to hydroxyl replacement—particularly after the 30-day period. The effect of iron was definitely shown only in the Cecil clay. Except in the 1 and 10 p.p.m. P solutions, where it was evident as a maximum at about pH 4.5, fixation due to iron was not distinguishable from that due to hydroxyl replacement. The clay containing the free iron oxides fixed a larger amount of phosphate in all cases than did the corresponding treated clay.

Montmorillonite (bentonite) showed fixation apparently due to aluminum at both phosphate concentrations employed (1 and 100 p.p.m. P in solution). Fixation by aluminum and iron was probably operative in the case of illite, but the curves obtained did not give definite indications.

The study of phosphate fixation in equilibrium systems at different pH values was supplemented by experiments in which the phosphated clays were leached with water and the amounts of phosphate extracted

were determined. The large amounts of phosphate retained by Cecil clay and kaolinite samples, presumably held largely by the process of hydroxyl replacement, showed a rather high rate of release, indicating that a considerable portion of the phosphate could be used by plants. A comparison of the Cecil clay treated to remove free iron oxides with the clay containing the free oxides showed that considerably larger amounts of phosphate were retained after leaching where the clay contained the free oxides. The much narrower ratio of the amounts of phosphate fixed by the two samples in an equilibrium system suggests that a large portion of the phosphate held by hydroxyl replacement in an equilibrium system was readily removed by washing with water and that a large part of the phosphate held after the untreated clay had been washed with water was retained by the free oxides. It was considered, however, that the kaolinitic portion of the Cecil clay can hold some phosphate in a form of low solubility. Leaching bentonite with water removed most of the fixed phosphate. Saturated $(\text{NH}_4)_2\text{C}_2\text{O}_4$, adjusted to pH 7 rapidly and almost completely extracted the phosphate fixed by the bentonite.

Greenhouse experiments with mixtures of sand and some of the clays after addition of various amounts of phosphate showed that little of the phosphate held by bentonite or by kaolinite finer than 2 micron in diameter was fixed in a form unavailable to tomatoes. The Cecil clay samples fixed a considerable amount and the finely ground kaolinite fixed most of the added phosphate, as indicated by the growth of tomatoes. The fact that tomatoes grew less vigorously on the Cecil clay treated to remove free iron oxides than on the corresponding untreated sample, was taken as an indication that the free oxides fixed the phosphate first and held it in a form more available than the phosphate which had contacted only the kaolinitic portion of the clay.

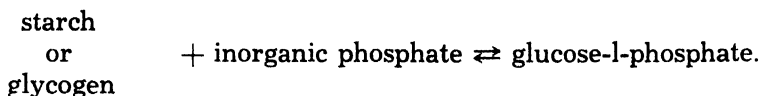
As a result of the experiments with kaolinite and the Cecil clay, it was concluded that under the proper conditions the kaolinitic portion of the Cecil clay can retain extremely large amounts of phosphate by hydroxyl replacement, but that combination with the free oxides is of much greater importance in determining the concentration of phosphate in the soil solution.

A STUDY OF THE PHOSPHORYLASE OF WAXY MAIZE¹

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A recent development in the investigation of carbohydrate metabolism has been concerned with the reversible reaction,



Phosphorylase, the enzyme which catalyzes this reaction, has been found in extracts of yeast, in such animal tissues as heart, brain, muscle, and liver, and in several plants, principally peas and potatoes. The present work dealing with the phosphorylase of waxy maize was designed to suggest a basis for explaining the role of enzyme action in the formation of starch in the corn kernel and to determine the usefulness of waxy maize as a source of phosphorylase.

For testing the activity of the enzyme, determinations of inorganic phosphorus and ester phosphorus in digestion mixtures were made by the method of Fiske and Subbarow (1), as modified by King (2). The intensity of the color in the molybdenum blue solutions was measured with a photoelectric colorimeter. A study of the method led to the standardization of the procedure which was used for all determinations.

Preliminary experiments based upon the work of Hanes (3, 4) and of Cori and co-workers (5) were made to ascertain the factors which effect the activity of the phosphorylase system of waxy maize. Those reported were concerned with the substrate for enzyme action, the age of the corn used as a source of enzyme, the treatment of the enzyme extracts by dialysis or by adsorption of the enzyme on $\text{C}\gamma$ aluminum hydroxide, and the clarification of the enzyme extracts with kaolin. Thus these preliminary experiments formed a basis for further studies on the concentration of the enzyme and the methods used for determination of phosphorylase activity, and for an investigation of starch synthesis through the reversible reaction previously indicated.

For use as a substrate in testing the activity of the enzyme, synthetic glucose-1-phosphate was prepared. The method used was that worked out by Cori *et al.* (6). α -Acetobromoglucose was treated with silver phosphate, the intermediate product hydrolyzed in hydrochloric acid, and the barium salt of glucose-1-phosphate precipitated in ethanol. The barium salt was then converted to the dipotassium salt, a crystalline dihydrate with a specific rotation of $+79^\circ$.

Activity tests were run on waxy maize at different stages of development. The phosphorylase unit, defined by Green and Stumpf (7) as the

¹ Original thesis submitted June 1, 1943. Doctoral thesis number 719.

amount of enzyme necessary to catalyze the liberation of 0.1 mg. of inorganic phosphate from glucose-1-phosphate in 3 minutes at 38° and pH 6.0, was used as a basis for these tests. It was found that the number of units per gram of dry corn varied from 5.09 to 0.28 during the period from 2 weeks after pollination to maturity. Calculated on the basis of the weight of corn as collected from the field, the activity varied from 0.85 to 0.26 units over the same period. It was concluded that phosphorylase is most abundant in the early corn, when starch synthesis is first starting, and that the concentration of the enzyme decreases as the corn kernel develops.

Concentration of phosphorylase from waxy maize was brought about by precipitation with ammonium sulfate, as worked out for the potato enzyme by Green and Stumpf. In the use of ammonium sulfate as the precipitating agent, it was necessary to determine the concentration that would give an enzyme precipitate in which the greatest part of the original activity of the extract was retained and which held only a relatively small amount of inactive material. It was determined that a fraction of low activity could be removed by 0.28 saturation with ammonium sulfate, leaving 78 per cent of the original activity in solution. This solution was then brought to 0.34 saturation with ammonium sulfate, thereby forming a precipitate which represented the protein insoluble at 0.28 to 0.34 saturation. This precipitate contained the highest concentration of phosphorylase and could be dissolved in a small amount of citrate buffer to give a preparation of three times the concentration of the original extract.

An investigation of the polysaccharide synthesized by the action of waxy maize phosphorylase was undertaken in order to determine the nature of the linkages in the synthetic product and thus to compare it with the natural starch from the same source. Bates and co-workers (8) found that a potentiometric iodine titration yielded information regarding the structure of the polysaccharide. By such a titration Bates *et al.* could estimate the amount of each of the two starch fractions, amylose and amylopectin, present in the sample. When amylose was titrated, an iodine complex was formed, and the iodine activity remained about the same until the complex formation was completed, when the potential began to rise sharply. Amylopectin, on the other hand, showed a steady increase in potential throughout the titration.

This technique was applied to the analysis of the small amounts of synthetic polysaccharide formed in digests containing waxy maize phosphorylase. The results indicated that during short periods of digestion at room temperature small amounts of straight-chain amylose were formed. Apparently, the larger proportion of the synthetic product in such cases was amylose of varying chain-lengths. During digestion at a higher temperature or with more active enzyme, larger amounts of polysaccharide were synthesized. However, the greater part of this polysaccharide was of the amylopectin, or branched type. It was concluded from these observations that short amylose chains might be formed first in the synthesis

and that many of these short amylose chains might later be brought together, forming a branched structure.

The iodine titration offered a clue to the nature of the phosphorylase present in waxy maize. Meyer (9) has suggested the existence of two such enzymes, one of which synthesizes α -1, 4-, or straight-chain, linkages, and the other of which synthesizes α -1, 6-, or branched-chain linkages. A phosphorylase of the 1,4-type would form amylose only, whereas both types of enzyme would be required for the formation of amylopectin, which contains both types of linkages. It has been shown that many phosphorylase extracts (*in vitro*) synthesize amylose only and thus contain only 1,4-phosphorylase. Since the 1,6-phosphorylase is assumed to be present *in vivo* in all natural sources (because all native starches contain some amylopectin), it is concluded that the 1,6-enzyme may be lost during the extraction processes. From the results of the present experiments, it was concluded that the 1,6-enzyme, as well as the 1,4-phosphorylase, was present in the waxy maize enzyme extract.

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A STUDY OF CYANAMIDE AS A SOLVENT AND REACTION MEDIUM¹

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Cyanamide has been known as a specific compound since 1851. Many methods of preparation have been published, and the physical properties of the compound have been determined. Its chemical reactions have been studied, and many compounds have been prepared. However, no investigations have been made of the properties of cyanamide as a solvent, as a reaction medium, and as an ionizing medium. In the present research these properties were investigated.

Part of the cyanamide used in this investigation was purchased from the Eastman Kodak Company, Rochester, New York. Some was made by the method of Werner (*Jour. Chem. Soc.*, 109, 1325-1327 (1916)). The melting point was 41°-42° C.

The solubilities of seventy-five organic and inorganic compounds were determined. The solid cyanamide was quickly placed in a 4 ml. test tube which was stoppered and placed in a water bath. The bath was kept at a temperature of 54°-55° C. by means of a Cenco-Dekhotinsky thermoregulator. The cyanamide melted, and the liquid was used as a solvent. From 0.5 to 0.75 ml. of the liquid was used for each test.

If the substance to be tested was a liquid, it was added a drop at a time. If as much as 0.25 ml. dissolved, the substance was considered as soluble; if one drop produced two layers, it was classified as insoluble. In the case of solid substances, from 0.05 to 0.07 g. of the solid was added in small portions. If this amount dissolved, the solid was considered as soluble. If less than half this quantity dissolved, the compound was classified as slightly soluble.

In general, those inorganic compounds which have a high solubility in water were found to be soluble in cyanamide. Comparatively few organic compounds were tested, but certain acids, alcohols, and esters were found to be soluble. These organic compounds had low molecular weights.

Liquid cyanamide proved to be a good reaction medium. When a solution of silver nitrate in the liquid was added to cyanamide solutions of soluble chlorides, bromides, iodides, carbonates, and phosphates, precipitates were produced immediately. The colors of these precipitates were the same as those of silver salts precipitated from aqueous solutions.

Antimony chloride formed a white crystalline precipitate when added to liquid cyanamide. This precipitated compound was insoluble in absolute alcohol and ether and could be dried in an oven at 65° C. The compound was analyzed for antimony by oxidation with iodine; for

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nitrogen by the micro Dumas method; for chlorine by the Volhard method. The results of the analyses gave an average of 43.98 per cent antimony, 29.37 per cent nitrogen, and 15.36 per cent chlorine. The calculated percentages for the compound $\text{Sb}(\text{HN.CN})_3.\text{HCl}$ are: antimony, 43.29; nitrogen, 29.87; chlorine, 12.61.

When anhydrous cupric sulfate was added to cyanamide, the salt became dark green, which indicated the formation of a solvated compound. Several samples of the green compound were prepared. The excess cyanamide was removed with anhydrous ether and the compound dried in a vacuum desiccator.

The compound was found to be very hygroscopic and could not be exposed to the air. Samples were quickly transferred to a small weighing bottle with a ground glass stopper. After weighing, the samples were ignited to cupric oxide. The oxide, after weighing, was dissolved in dilute nitric acid, and copper was determined by the potassium iodide method. The results indicated the formation of $\text{CuSO}_4.2\text{H}_2\text{NCN}$ in some samples and $\text{CuSO}_4.3\text{H}_2\text{NCN}$ in others.

A mixture of dry hydrogen chloride and nitrogen was passed into liquid cyanamide at 55°C . A white crystalline compound formed which could be washed with ether and dried in an oven at 65°C . The compound was stable under room conditions. It was analyzed for chlorine by the method of Volhard. The average percentage of chlorine was found to be 43.51. The calculated percentage of chlorine in the compound $\text{H}_2\text{N.CN.HCl}$ is 45.20. The fact that the percentage of chlorine in the samples analyzed was low could be accounted for by some polymerization of cyanamide to dicyandiamide. The polymer will not react with HCl and is only slightly soluble in ether.

When potassium phosphate was added to cyanamide, the solution became viscous. Upon continued addition of the salt, a white crystalline compound separated. This was washed with absolute alcohol and then with ether and dried in an oven at 65°C .

This compound, when dissolved in water, gave only faint tests for phosphate with silver nitrate, magnesia mixture, and molybdate reagent. Its melting point was 200°C . by the micro method. Analyses for nitrogen by the micro Dumas method gave an average of 66.61 per cent. These data indicated dicyandiamide contaminated with a slight amount of potassium phosphate.

Some conductivity measurements were made with solutions of ammonium chloride, ammonium nitrate, potassium iodide, potassium phosphate, and sodium nitrate. Reagent grade salts were used and dissolved in liquid cyanamide at a temperature of 54° – 55°C .

The apparatus used in this part of the investigation consisted of a conductivity cell, resistance box, ear-phones, and Wheatstone bridge. The current was generated with an oscillating circuit composed of radio tubes with proper capacities and inductances.

The cell was of standard type but was built to have a capacity of about 5 ml. It consisted of two circular platinum plates set vertically in

a cylindrical cell. Connection was made through platinum wire sealed through the glass and leading into wells containing mercury. The electrodes were platinized.

The cell was calibrated at 55°C. and found to contain 4.30 ml. The cell constant was determined by using a 0.02 molar solution of potassium chloride.

About 4.00 ml. of cyanamide were placed in the cell and the specific conductivity measured. A weighed amount of salt was introduced and the volume brought up to 4.30 ml. by the addition of cyanamide. The specific conductivity of the solution was determined. Then one-sixth of the original volume of the solution was removed, and an equal amount of pure cyanamide was added. The specific conductivity of this more dilute solution was then measured. The solutions were progressively diluted until at least seven different concentrations were obtained for each solute.

The initial concentrations of solutions ranged from 0.0097 to 0.3283 normal. For every dilution tested, the solution showed an increase in specific conductivity over that of the pure solvent.

Graphs prepared by plotting the volume in liters containing one gram-equivalent of salt against the equivalent conductance gave irregular curves. In the case of potassium iodide, the solutions showed an increase in equivalent conductivity with progressive dilution. Other series of solutions, except potassium phosphate, gave an initial rise and then a decrease in equivalent conductivity.

Potassium phosphate solutions showed a steady decrease in equivalent conductivity for eight dilutions. This was due to polymerization of the cyanamide to dicyandiamide. Since it can be shown that potassium phosphate causes polymerization, other salts must have the same effect. The irregular form of the dilution curves must be due to this effect.

ECOLOGY AND MANAGEMENT OF THE PRAIRIE SPOTTED
SKUNK, *SPILOGALE INTERRUPTA* (RAFINESQUE),
IN SOUTHEASTERN IOWA ¹

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The prairie spotted skunk, *Spilogale interrupta* (Rafinesque), was studied in southeastern Iowa at Stockport from March, 1939, to June, 1942, with particular reference to its ecology, life history, and management.

The Stockport skunk research area consists of 17.5 square miles, approximately 7 in rough woodland pasture and 10 in flat cultivated farm land on which the research was largely done.

Live trapping and ear-tagging for release and future capture was the basic method employed in studying the range and ecological requirements of the population. Four central sections were selected for intensive studies and subjected to rotational trapping. One section was trapped at a time with one trap on each of sixty-four quadrats of 10 acres each.

The traps were moved to new quadrats once every 3 weeks and rotated in such a manner that the central sixty-four quadrats received twice as much trapping (6 weeks) as the bordering quadrats (3 weeks) each season of 3 months.

Ground cover on these four sections during the first part of 1942 was cornstalks (20 per cent), bean stubble (11 per cent), pasture (26 per cent), clover (14 per cent), farm yards (3 per cent), woodland (5 per cent) and several other crop residues each occupying less than 3 per cent.

A major objective of the investigation was to uncover some of the facts concerning territories and the constituents of the habitat of the spotted skunk. The habitat was characterized by a large number of dens distributed over the range. These dens were not the property of any one individual but of the population. Sixty dens were used from one to five months on the four sections under intensive observation during the winter and spring of 1942. Ninety per cent of these dens were directly related to farm buildings and crop accumulations associated with agricultural practices of the region.

Several important features were found essential to continual den usage. The exclusion of light was probably most important. Without exception every den or semblance of a den met this requirement. A second important feature was protection against weather conditions. Summer heat, winter cold, snow, rain and cold wind all were factors influencing den usage at varying times. A third important requirement was protection against "enemies," especially farm dogs and men.

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An expression "area of familiarity" was used to describe the living space of individual skunks. Only a part of the area of familiarity was used by individual spotted skunks at any one time. When any part no longer provided the necessities of life, the skunk moved to portions previously used or extended the area of familiarity to new ground. The fact that one skunk moved in on another seemed to make no great difference. A place to sleep reasonably secure from dogs, man, daylight, and adverse weather; availability of any of a large number of foods—animal or plant; and a reasonably safe passageway between dens and food were basic factors regulating the limit of usefulness of any part of the area of familiarity.

A survey of seventy-eight farm yards on the whole area revealed that 60 per cent of the farm yards had three or more permanent or semipermanent den sites, and 30 per cent had at least two semipermanent or permanent den sites.

The population density was determined at thirteen skunks per square mile with a sex ratio of 1.68:1.

Home ranges of males during winter were restricted to 160 acres or less. Individuals had their dens in hay barns or similar buildings and found their food in the vicinity. They ordinarily had two or more permanent or semipermanent stopping places or nests in the vicinity of their central den, and among these places they ate and slept. Eight males were followed closely during January and February of 1942 on the central four sections. Six of these were known to be regularly using barns for basic dens, and in the vicinity of these barns they were regularly caught. During good weather they often made foraging excursions along fence lines and into the surrounding fields.

During the winter season only one or two records were ever obtained of an animal traveling more than $\frac{1}{2}$ mile, and these never exceeded $\frac{3}{4}$ of a mile from a central winter den.

Trapped females in winter presented the same or similar activity pattern demonstrated by trapped males. Six individuals were followed by repeated trappings during January and February of 1942. They made similar one night forays into the surrounding fields during good weather and were to be found in the vicinity of a barn den (five cases) or straw-pile den (one case) to which they returned for the day. During wet or very cold weather they did not go out of the barn dens.

Data on spring activity of males were gathered on eighteen individuals using the four sections. Males at this time of the year traveled about the community. Distances of $\frac{1}{2}$ mile to a mile were regularly traveled between points of capture, and two of them were caught at points 2 miles apart during this time. Six individuals traveled about extensively within the bounds of the 4-square-mile area, and from these it is assumed that from 2 to 4 square miles was the size of the spring range and probably the extent of their area of familiarity.

The area used by females was in marked contrast with the size of that used by males during the spring season. While the males were

moving all over the community, the females changed their travel habits only a little or not at all. None of the cases demonstrated the use of area greater than one-fourth section.

All available data indicated that summer and fall activities of both sexes were similar to those demonstrated during the spring season.

Seventy-seven spotted skunks were killed on the area: 32 per cent for fur, 26 per cent by dogs, and 27 per cent by men for suspected predation on chickens or for living in houses. Mortality was greatest during winter and least during summer.

Eighty-five per cent of the farmers on the area had had the spotted skunks about their farm yards during the period from June, 1941, to June, 1942. Thirty-four per cent had had trouble with them. Twenty-one per cent attempted to manage them. Twenty-eight per cent encouraged their presence about the premises, 19 per cent were definitely against having them about their premises, and 50 per cent were apathetic toward them.

A litter of seven spotted skunks was observed in captivity to determine the rate of growth. Each weighed approximately 10 grams at birth. Their eyes opened at 31 days. Their teeth cut through the gums at 35 days. They walked on their feet at 35 days. They were weaned, or nearly so, at 54 days. They appeared full grown at 104 days.

Weights were obtained on 151 spotted skunks in all seasons. Males averaged 1 pound, 9½ ounces; females averaged 1 pound, 1 ounce.

Of six spotted skunks transplanted, two transplants were known to have been successful.

Encouragement and discouragement types of management were practiced on the research area by farmers. Spotted skunks were encouraged for their possible controlling effect upon rodent population and discouraged for predation on poultry or for making dens in houses, wells, and among stored feed where their presence was not conducive to human well-being.

Wise management of spotted skunks is co-ordinated with many good farming practices. In general spotted skunks deserve encouragement about Iowa farms.

THE CHEMICAL RESISTANCE OF POLYHEXAMETHYLENE ADIPAMIDE¹

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A sulfur-free woven nylon, polyhexamethylene adipamide containing 0.28 per cent of titanium dioxide and yielding 0.35 per cent of ash, was studied in an attempt to determine quantitatively its resistance to mordanting, heat, and light.

When the fabric was mordanted with 50-volume baths of aluminum sulfate, containing 0.40, 0.50, 1.50, 3.00, and 6.00 g. of aluminum oxide per gram of fabric, for 1 hour at 100°C., a maximum of but 0.13 per cent of Al_2O_3 was fixed from the mordanting bath of 0.50 g. of aluminum oxide per gram of nylon at a pH of 3.00. The fixation of aluminum which, though slight, increased with an increase of pH is in accord with the electrostatic theory of mordanting, but the data suggest that the isoelectric range of this oriented nylon is nearer a pH of 3.00 than a pH of 2.7.

The effect of 50-volume baths of potassium dichromate in 1 hour at 100°C. on the weight, chromic oxide, total nitrogen, and wet strength of the fabric was investigated. As the concentration of potassium dichromate was raised from 0.0207 to 0.1278 g. of Cr_2O_3 per gram of fabric, the pH of the solution and the percental exhaustion decreased, the amount of ash increased slightly, and the wet strength was not changed. As the pH of a solution containing 0.0517 g. of Cr_2O_3 per gram of fabric was decreased by the addition of hydrochloric acid, the percentage of ash and exhaustion increased, and the wet strength decreased. A straight-line relationship was shown between the Cr_2O_3 fixed by the fabric and that left in the mordanting bath. No change in slope of this line occurred at the isoelectric region of the fiber nor where the chromium changed from one ionic combination to another. Fabric treated with potassium dichromate of pH 0.85 lost 59 per cent of its wet strength but retained its original weight and amount of nitrogen. This retention is evidence that hydrolysis of the amide linkage, if it occurred, was random. During 1 hour at 100°C. in mordanting baths of pH less than 1.5, the fabric lost more than 38 per cent in wet strength.

Dry heat for 2 hours at 134 to 137°C., as well as boiling water for 1 hour, each effected a decrease of but 9 per cent in the wet strength of the fabric. The wet strength was not changed by ironing at temperatures up to 197°C., but the fabric yellowed and shrank 6 per cent when ironed between 184°C. and 197°C.

Fabric treated with steam at 138°C. for 5 minutes decreased 15 per cent in wet strength; that treated with steam at 148°C. showed a loss in

¹ Original thesis submitted December 10, 1942. Doctoral thesis number 698A.

wet strength of 26 per cent but no loss in weight or nitrogen; and steam at 154°C. effected a decrease of 35 per cent in wet strength and a shrinkage of 6 per cent. Again in this instance, if hydrolysis of the amide linkage occurred, it was random.

After exposure to ultraviolet light for 45 and for 60 hours, the original fabric decreased 26 and 53 per cent in wet strength. The fabric was treated with alkali alkyl sulfate, calcium hypochlorite, formaldehyde, iodine, phenol, potassium dichromate, potassium permanganate, sodium hydroxide, and sulfuric acid. Alkali alkyl sulfate and formaldehyde at 25°C. effected an increase of 6 per cent in wet strength; calcium hypochlorite, iodine, potassium dichromate, potassium permanganate, and sodium hydroxide each at 25°C. did not affect the wet strength of the fabric whereas phenol and sulfuric acid effected a slight loss. Fabric treated with solutions of potassium permanganate containing added acid or alkali lost 16 to 22 per cent in wet strength. Fabric treated with alkali alkyl sulfate, calcium hypochlorite, formaldehyde, potassium dichromate, and with sodium hydroxide was also exposed to ultraviolet light for 45 hours. Treatment of the fabric with 0.2906*N* calcium hypochlorite or with 0.1000*N* or 0.2000*N* sodium hydroxide for 2 hours at 25°C. provided some protection to degradation by ultraviolet light as measured by its wet strength. Upon exposure to ultraviolet light for 45 hours, fabric treated with 0.25 per cent alkali alkyl sulfate, 1 per cent formaldehyde, or 0.1000*N* potassium dichromate lost 47, 38, or 50 per cent of its wet strength.

The woven nylon has been shown to be resistant to oxidants at room temperature, to be less resistant to oxidants in the presence of an inorganic acid, to be quite resistant to dry heat but not resistant to saturated steam at a temperature greater than 138°C., and to be degraded appreciably with ultraviolet.

THE DATA, DESIGN, AND SPECIFICATIONS FOR A PLANT TO PRODUCE XYLOSE FROM CORNSTALKS¹

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In recent years, considerable research has been directed toward the utilization of agricultural waste products. The Department of Chemical Engineering and the Engineering Experiment Station of Iowa State College have done a great deal of work on developing uses for agricultural wastes, particularly cornstalks and corncobs. Among the products that have been studied is the interesting sugar, xylose, the product of mild hydrolysis of the pentosan fraction of the plant material. Until recently, xylose has been classed as a rare sugar and has been produced in very small quantities for use in bacteriological investigations and other fields of research. However, in view of the many possible uses for the product, it seemed advisable to develop a process for producing it on a large scale from cornstalks.

Detailed studies were made to determine the proper conditions for hydrolysis. Sulfuric acid was found to be most satisfactory as a catalyst for the process, and a ratio of cornstalks to acid solution of 0.10 gave the best results. In order to arrive at the optimum conditions of pressure, time of hydrolysis, and acid concentration, a number of tests were made in which one variable was allowed to vary over a definite range while the others were kept constant. The yield of xylose from each test was determined by the standard iodimetric method of analysis. Upon examining the data obtained in this way, it was possible to arrive at the optimum conditions for carrying out the process. It was found that there were several combinations possible:

Pressure p.s.i.	Acid Concentration N.	Time hours
20	0.40	2.0
30	0.20	2.0
40	0.20	1.0
40	0.10	2.0

After the optimum conditions for hydrolysis had been decided upon, the next step was to examine methods of removing the xylose extracts from the cornstalks. Several methods were studied, and the counter-current method of extraction was found to be best adapted to this process. One acid hydrolysis was found to be sufficient for each batch of corn-

¹ Original thesis submitted December 15, 1942. Doctoral thesis number 704.

stalks; and two washes, after the original extract had been drained, were found to be adequate. In the countercurrent system of washing, the first washing from each batch was used in making up the following batch for hydrolysis. Enough sulfuric acid was added in each case to make up the desired acid concentration. The second washing from each batch was used for the first washing of the next batch. By continuing in this manner, xylose concentrations were built up from 3.61 per cent for the first batch to 5.07 per cent for the fifth.

Various methods of purifying the syrups were studied in order to produce syrups which could be crystallized. Best results were obtained by using a combination treatment with phosphoric acid and activated carbon.

The process which was used in producing xylose from cornstalks on the pilot plant scale, is described as follows: Baled cornstalks were brought from storage and were shredded in a rod mill. As they were discharged from the rod mill, they passed through a trommel screen where they were washed free of dirt and water soluble materials. The clean shredded stalks were then transferred to a pressure cooker, covered with water, and digested under a pressure of 20 pounds per square inch for 2 hours. After the digestion, the cooker was "blown off" and the liquid was allowed to drain. The stalks were washed thoroughly with cold water, and were removed from the cooker. In order to remove the excess water present, they were then pressed in a hydraulic press and were dried in a cabinet drier. Following the above treatment of the stalks, hydrolysis was carried out by cooking them with 0.2 normal sulfuric acid at 40 pounds per square inch pressure for 1 hour. The extract from the acid hydrolysis was drained off, and the stalks were washed using the countercurrent method as described before. Calcium carbonate was then added slowly to the acid extract until the pH became 5.2, and the calcium sulfate formed was filtered off. The sugar solution was given a treatment with partially spent activated carbon to remove objectionable impurities, and was then concentrated by evaporation in a vacuum evaporator to a specific gravity of 1.2 (measured at 20 degrees centigrade). Ortho phosphoric acid was added to this syrup until the pH became 4.0. A flocculent precipitate formed which carried down a considerable amount of the impurities present. The syrup was then filtered to remove this precipitate together with the calcium sulfate which had precipitated during the evaporation. At this point the syrup was given a second treatment with fresh activated carbon to remove color produced by caramelization in the evaporator. Further concentration was carried out in a small evaporator to a specific gravity of 1.36 (measured at 50 degrees centigrade), and the syrup was then placed in a crystallizer, and the temperature was lowered gradually until crystallization occurred. The crystals were then separated, washed, and dried in an oven at low temperature.

Using data obtained in the operation of the pilot plant, a large plant was designed to produce 1,000 pounds of xylose per day. The capital

investment required for this plant is \$104,742. By cost accounting this plant, it was found that the cost of producing the xylose from cornstalks was 18.8 cents per pound in the purified syrup form and 24.7 cents per pound in the crystalline form. Allowing 30 per cent for distribution, and profits, the xylose as syrup could be sold for 27 cents per pound and the crystalline xylose for 35 cents per pound.

THE DETERMINATION OF ETHYL ALCOHOL IN THE BLOOD AND TISSUES, ITS ABSORPTION AND DISTRIBUTION, AND ITS EFFECT UPON SOME OF THE BLOOD CONSTITUENTS OF THE RAT¹

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A search of the literature revealed no method which was convenient for the determination of alcohol in the blood of rats in the experiments which were to be made. Several methods were devised for the proposed work.

A micro method was developed for the determination of alcohol in the blood using samples of 0.1 or 0.05 ml. of freely flowing or oxalated blood.

The blood sample was diluted with about 10 ml. of distilled water and placed in a 50 ml. distillation flask of the Kjeldahl type. One ml. of a 10 per cent solution of sodium tungstate and 1 ml. of a mercuric sulfate-sulfuric acid solution were added in order. The mixture was then distilled, and the first 5 ml. of distillate were collected in a 22 × 175 mm. pyrex test tube. To this distillate were added 1 ml. of 0.0434 *N* potassium dichromate and 5 ml. of concentrated sulfuric acid. The solution was thoroughly mixed and allowed to stand for 10 minutes or more. The excess dichromate was then titrated with a solution of methyl orange and ferrous sulfate in 60 per cent sulfuric acid. A blank determination was made using 5 ml. of water instead of the distillate. After a sample or the blank had been titrated to the end point, 1 ml. of the standard dichromate was added and the solution again titrated to the end point. The latter titration serves to standardize the reducing solution. The blank corrects for reducing substances present in the sulfuric acid. One ml. of 0.0434 *N* dichromate is equivalent to 0.5 mg. of ethyl alcohol. From these data the alcoholic concentration of the blood may be calculated.

The apparatus used in this method has been described and is very convenient. The method gave very accurate results and good recovery of alcohol from standard solutions. The preparation of these standard alcohol solutions has been described.

A method was developed for the analysis of tissues for alcohol. The tissues were removed and placed in weighed 150 ml. extraction flasks. A small piece of solid carbon dioxide was placed in each flask to cool the tissue. The flasks containing the tissue were weighed again after reaching room temperature. The tissues were covered with a solution of 10 per cent tartaric acid in half saturated picric acid and then were stored in a refrigerator until the analysis could be completed.

The alcohol was removed by steam distillation using a special apparatus. The apparatus was so constructed that the tissue could be

¹ Original thesis submitted December 15, 1942. Doctoral thesis number 703.

minced and the alcohol removed by steam distillation without removing the tissue from the flask. The distillate was collected in a volumetric flask of such a size that an aliquot of 5 or 10 ml. contained not more than 0.45 mg. of alcohol. The aliquot was diluted to 10 ml. and treated with sodium tungstate and mercuric sulfate-sulfuric acid solutions as in the analysis of blood. The procedure used in the remainder of the analysis was the same as that used for blood.

Micro modifications were made of standard methods used in the analysis of blood for glucose, uric acid, and nonprotein nitrogen. The methods were modified in order to carry out the determination using 0.1 ml. or less of blood.

A series of experiments was conducted in order to determine the distribution of alcohol in various tissues following the oral administration of 2.5 grams of alcohol per kilo body weight to fasted rats. The distribution was studied 30 minutes, 2 hours, and 4 hours following the administration of the alcohol. The amount of substances present in the tissues of rats receiving no alcohol, which reacted as alcohol in the determination, was determined.

Since the amount of alcohol present in the tissues depends upon the amount of absorbed alcohol, the actual concentration in the tissues depends upon the rate at which it is absorbed from the digestive tract. The rate of absorption varied between individual animals, hence the actual amount of alcohol in the tissues also varied. The ratio of the concentration of alcohol in the tissues to that in the blood was quite constant in the various cases studied. This ratio remained almost constant during the period of time from 1.5 to 3 hours following the oral administration of 2.5 grams of alcohol per kilo to fasted rats. The ratios found for that period for some of the tissues studied were as follows: lungs and spleen, 0.80; kidneys, 0.75; liver and muscle, 0.70; brain and heart, 0.60; testes, 0.55; pancreas, 0.50; and bone, 0.30.

Most of these ratios had decreased somewhat at 4 hours following the administration of the alcohol. Those tissues which showed the most constant ratios after the distribution of the alcohol was complete were: the spleen, kidneys, muscle, and lungs.

The absorption of alcohol into the blood following the oral administration of 2.0 and 2.5 grams of alcohol per kilo body weight was studied. These experiments were made using fasted and unfasted rats. The effects of habituation to alcohol were studied, and it was found that habituation had no influence upon the absorption of alcohol from the digestive tract. Average blood alcohol curves were obtained. It was found that 2.5 grams of alcohol per kilo occasionally caused pylorospasms which delayed the absorption of the alcohol. Average curves were given excluding those rats which showed pylorospasms while other average curves were given including all rats studied under similar conditions.

The influence of some substances administered with alcohol upon the absorption of the latter into the blood was studied. Whole milk, skim milk, cream (50 per cent butterfat), and glucose were studied. Whole

milk and cream had a marked inhibitory action on the absorption of the alcohol from the digestive tract. The other substances studied had very little or no effect.

Various amounts of alcohol were injected intraperitoneally, and the resulting concentration of alcohol in the blood was determined. It was shown that a maximum concentration of alcohol in the blood was reached within 15 to 20 minutes following the injection of the alcohol. The administration of 1.86 grams of alcohol by injection was the highest level of injection which could be easily studied. The injection of 2.5 grams of alcohol per kilo caused the development of coma and a fall in blood pressure which made it difficult to obtain satisfactory samples.

The influence of continued ingestion of alcohol upon growth and feed consumption was studied. Alcohol solutions containing 1.0, 5.0, and 10 per cent alcohol were compared with distilled water in the growth and feed consumption studies. The ingestion of 10 per cent alcohol caused a diminished growth rate during the first month of the experiment. The other solutions showed little effect upon growth. The continued ingestion of 10 per cent alcohol over a period of several months resulted in a poor nutritional state of the female rats studied.

It was found that no changes followed the ingestion of 10 per cent alcohol in the concentration of hemoglobin, uric acid, or nonprotein nitrogen. There was, however, a slight decrease in blood sugar concentration following a 36-hour fasting period. This is explained by the fact that the ingestion of alcohol causes a fatty infiltration of the liver. This causes a diminished glycogen store, hence a lowered blood sugar level following a fasting period of 36 hours.

THE EFFECT OF VARIATION IN MASHING PROCEDURES UPON THE ALCOHOLIC FERMENTATION OF CORN¹

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INTRODUCTION

The production of ethanol by fermentation has become of vital importance in this nation's war effort. Enormous quantities of ethanol are used or will be used in the manufacture of munitions and synthetic rubber. The chief raw materials for fermentation now available in the United States are the grains, since blackstrap molasses, which was formerly most extensively used, is no longer imported in large quantities because of shipping restrictions. Before grain can be fermented by yeast, the starch must be converted to sugars. Enzymes, either from barley malt or from mold preparations, may be used to bring about this saccharification. The treatment of the grain mash before and during saccharification affects considerably the final yield of ethanol, and various methods of handling the mash prior to fermentation constituted the subject of this investigation.

METHODS

Variations of three methods of preparing corn mashes for fermentation were investigated. The first method was rapid cooling of the cooked mashes from 100° C. to 55° C. by the addition of cold water containing the saccharifying agent. The mashes were saccharified at 55° C. for lengths of time varying from 2 minutes to 3 hours. The second method was a two-stage procedure, and consisted of thinning the mash by adding a small portion of the saccharifying agent at 70° to 80° C., followed by saccharification at 55° or 30° C. by a second portion of saccharifying agent. The third method involved the use of hydrochloric acid or sulfuric acid for thinning the mashes. Twenty-two per cent mashes were cooked with 0.04 normal acid for 1 hour at a steam pressure of 15 pounds per square inch. Fifty per cent mashes were cooked with 0.08 normal acid under the same conditions. The latter mashes were cooled rapidly from 100°C. to 55°C. by the addition of sufficient cold water to produce a final concentration of 22 per cent corn. The pH in each case was adjusted to 5.0 before saccharification. All mashes were treated with a saccharifying agent before being fermented.

In this investigation all mashes were cooked in an autoclave for 1 hour at a steam pressure of 15 pounds per square inch. Mashes were saccharified by malt and by mold-bran, which is an amylase preparation produced by growing a strain of *Aspergillus oryzae* on wheat bran. The mashes at the time of inoculation consisted of 50 grams of corn in 225 ml. of water. Each flask of mash was inoculated with 20 ml. of a 24-hour cul-

¹ Original thesis submitted May 29, 1943. Doctoral thesis number 717.

ture of a strain of *Saccharomyces cerevisiae*, and was then incubated at 30° C. for 80 to 90 hours. The ethanol content was determined by distilling the fermented mash and collecting the first 100 ml. of distillate. The specific gravity (25°/25°) of the distillate was determined and the ethanol content was read from tables. The ethanol yields obtained were calculated as the percentage of that theoretically obtainable from the starch originally present.

Comparative viscosities of representative mashes following saccharification were determined by measuring the drainage time of 100 ml. of the mash from a pipette with an enlarged tip. The drainage time divided by the drainage time for pure water was referred to as "specific viscosity."

RESULTS

Rapid cooling of the mashes resulted in an increase in ethanol yields of as much as 4.5 per cent in some cases when the mashes were saccharified by malt, and reduced the amount of malt required by about one-third. A saccharification period of 2 minutes at 55° C. was as satisfactory as a period of 1, 2, or 3 hours. Mold-bran was not as effective as malt for saccharifying rapidly cooled mashes. The quick-cooling process was the poorest of the three mashing procedures investigated. The mashes were not well thinned, and on fermentation gave comparatively low ethanol yields. The highest yield was 87.7 per cent of theory, from mashes saccharified for 1 hour at 55° C. by a malt concentration of 6 per cent of the weight of corn.

The two-stage mashing procedure was satisfactory when a small amount of malt was added to the mashes at 70° to 80° C. Two grams of malt per 100 grams of corn were better than 1 or 3 grams of malt. Mold-bran had no thinning effect when used in this manner. Best results were obtained when thinning took place at 75° or 80° C. Mashes thinned at 70° C. gave slightly lower ethanol yields upon fermentation, while those treated at 85° C. were quite viscous and fermented poorly.

Mold-bran was better than malt for the saccharification stage of two-stage mashing, and resulted in about 3 per cent higher yields of ethanol. A quantity of mold-bran equal to 5 per cent of the weight of the corn resulted in an ethanol yield of 91.5 per cent of theoretical. The best yield using malt was 88.2 per cent of theoretical, at a malt concentration of 8 per cent.

Both hydrochloric acid and sulfuric acid, at a concentration of 0.04 normal, thinned 22 per cent corn mashes readily upon cooking for 1 hour at 15 pounds per square inch steam pressure. Mold-bran was much better than malt for the saccharification of these mashes. Mashes thinned with 0.04 normal hydrochloric acid upon fermentation gave about 2 per cent higher yields of ethanol than did those thinned with 0.04 normal sulfuric acid.

Fifty per cent corn mashes which were cooked with 0.08 normal acid for 1 hour at a steam pressure of 15 pounds, and then diluted to a concentration of 22 per cent corn, were quite fluid. These mashes gave

better ethanol yields than did mashes thinned with 0.04 normal acids, and required less saccharifying agent. Rapid cooling of the acid-thinned mashes did not increase the ethanol yields. Mold-bran was superior to malt for the saccharification of mashes thinned with 0.08 normal acids. The highest ethanol yield obtained was 93.8 per cent of theory, from mashes thinned with hydrochloric acid and saccharified by 3.5 per cent mold-bran. Sulfuric acid was as effective as hydrochloric acid at a concentration of 0.08 normal, in the case of mashes saccharified by mold-bran. Hydrochloric acid was much the better for mashes saccharified by malt, and resulted in about 5 per cent higher ethanol yields.

Calcium carbonate, as well as sodium carbonate, was used to neutralize mashes thinned with 0.08 normal acids. Fermentation results with calcium carbonate as the neutralizing agent were equal to those with sodium carbonate in the case of mashes thinned with hydrochloric acid. Ethanol yields were slightly lower with calcium carbonate than with sodium carbonate as the neutralizing agent in the case of mashes thinned with sulfuric acid.

The highest ethanol yields obtained in this investigation, amounting to 93.8 per cent of theory, were from mashes thinned with 0.08 normal hydrochloric acid, adjusted to pH 5.0 with sodium carbonate solution, and saccharified by mold-bran. Yields were about 2 per cent higher than any produced by two-stage mashing, and about 6 per cent higher than the highest yield obtained from quickly cooled mashes.

No correlation was found between the specific viscosity of a saccharified mash and its fermentability by yeast.

CHARACTERIZATION OF COMPONENTS OF STARCH¹

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The heterogeneity of starch has long been recognized. However the numerous attempts to obtain definite fractionation have resulted in much confusion due, primarily, to the lack of methods for characterizing these fractions. The recent introduction of a branching concept for the starch molecule² has enabled Meyer³ to clarify the situation considerably. The hot water extraction of granular starch has been shown to yield a soluble component (amylose) consisting essentially of linear molecules and an insoluble component (amylopectin) which is apparently highly branched.

Two methods of fractionation have recently been developed which apparently give much sharper separations: (a) selective adsorption of the amylose component on cotton⁴ and (b) precipitation of amylose as a complex with n-butanol⁵. Furthermore, the development of an iodine titration method⁶ indicates amylose to be a definite and sharp component of starch and provides a method for its determination in any given starch or starch fraction.

Differentiation between these components on the basis of branching has been based principally on results of methylation and analysis of the methyl-glucoses arising upon hydrolysis. These results can be severely criticized inasmuch as the available methods for separating the methyl-glucoses are poor. Recent advances in the theory of solutions of high polymers⁷ suggest the possibility of utilizing solution viscosity and osmotic pressure studies in more thoroughly characterizing the components of starch. This mode of attack has been utilized in this investigation.

Osmotic pressure measurements were carried out on the acetates of corn amylose (prepared by the butanol method), corn amylopectin, waxy maize starch, tapioca amylose, and the β -amylase limit dextrin from waxy maize. Chloroform was used as solvent. The determination of osmotic pressures in organic solvents is attended with numerous experimental difficulties. Chief among these is the choice of a suitable membrane material. Several materials were tried in these studies, ordinary parchment proving most satisfactory. A simplified form of the Hepp micro-osmometer was designed and found to be fairly satisfactory; however, suggestions are given for its improvement.

¹ Original thesis submitted March 16, 1943. Doctoral thesis number 713.

² Staudinger and Husemann, *Ann.*, 527, 195 (1937).

³ Meyer, *Natural and synthetic high polymers*, pp. 387-417, Interscience Publishers, Inc., New York (1942).

⁴ Pacsu and Mullen, *Jour. Am. Chem. Soc.*, 63, 1168 (1941).

⁵ Schoch, *ibid.*, 64, 2957 (1942).

⁶ Bates, French, and Rundle, *ibid.*, 65, 142 (1943).

⁷ Mark, *Physical chemistry of high polymers*, Interscience Publishers, Inc., New York (1940).

The graph of P/C vs. C for corn amylose is linear with positive slope as predicted theoretically for a linear polymer. The molecular size as calculated from the limiting value of P/C is 250 glucose units. The same plot for corn amylopectin is much steeper and shows evidence of curvature so that the limiting value cannot be ascertained with certainty. However, the molecular weight is at least four times as great as that of corn amylose and probably much larger.

Solution viscosities were measured over the concentration range 0–0.8 per cent using anhydrous ethylenediamine as solvent. This is probably the best dispersing medium available for starch and its components. A few amylopectins failed to give clear solutions.

The viscosity-concentration behavior of the amyloses resembles very markedly that of known linear polymers giving added evidence of the linear nature of amylose. Synthetic starch shows increased polymer-polymer interaction, possibly due to the presence of polar groups in the molecule. Due to the method of synthesis (from glucose-1-phosphate) phosphate groups would be expected.

In spite of its much higher molecular weight corn amylopectin has only a slightly higher limiting viscosity than corn amylose. This suggests a more compact structure for amylopectin, probably branching. The steeper P/C vs. C plot would also be predicted on a theoretical basis for a branched molecule.

Construction of a Fisher-Hirschfelder model of amylose emphasizes its inability to kink randomly as do the simpler polymers. For this reason the Staudinger equation relating intrinsic viscosity and molecular weight cannot be expected to hold in this case. The equation

$$[\eta]_{sp}/C]_{c \rightarrow 0} = 0.07 + 1.2 \times 10^{-4} n^{1.6}$$

is suggested for the amylose series, n being the number of glucose units in the chain. The additive constant, 0.07, is equivalent to the Einstein constant for spherical molecules and was evaluated from the limiting viscosity of the Schardinger β -dextrin. This constant also corrects for solvation. The multiplicative constant takes care of the length of the chain per glucose unit while the exponential depends on the randomness of kinking.

The molecular weights of the various amyloses as calculated from

Material	Molecular Size (Glucose units)	Characteristic Potential (mv.)
Potato amylose	500	0.197
Tapioca amylose	450	.200
Lily amylose	310	.202
Corn amylose	250	.203
Corn "crystalline amylose"	175	.205
Corn amylose (hot water extraction method)	115
Synthetic starch	85	.204
Amylodextrin fraction No. 3	44	.218
Amylodextrin fraction No. 4	31

the above equation are summarized in the Table and compared with the potentials at which the materials take up iodine in the titration procedure⁶. There is obviously a close relationship between the affinity for iodine and the chain length. To explain this a new concept of the stability of the amylose-iodine complex is presented, viz., that it is due primarily to resonance interaction between iodine molecules oriented end to end in the amylose helices. This picture further explains qualitatively the intense blue color of the complex. Partial precipitation of amylose with iodine shows that the latter tends to saturate the amylose molecules successively rather than distributing uniformly as would be expected if the stability were due to interaction between amylose and iodine.

The variation in molecular size of corn amylose prepared by the various methods is of interest. Extraction with hot water removes preferentially the shorter chains as expected, while butanol precipitates the larger molecules. The "crystalline amylose," prepared by a combination of hot water extraction and butanol precipitation,⁸ has an intermediate molecular weight.

That waxy maize starch is essentially pure corn amylopectin is indicated by both its viscosity and osmotic behavior. The extremely low viscosity of the limit dextrin from waxy maize starch can be explained only on the basis of an essentially spherical molecule. Since the limit dextrin is apparently formed through the digestion of the free outer branches of the original material, this indicates waxy maize starch (and hence corn amylopectin) to have an essentially spherical, three-dimensional, netlike rather than herring-bone type of branched structure.

⁸ Kerr and Severson, *Jour. Am. Chem. Soc.*, 65, 193 (1943).

THE ISOLATION OF AEROBIC CELLULOSE-DECOMPOSING ORGANISMS AND THEIR ACTION ON CELLULOSE AND ASSOCIATED PLANT CONSTITUENTS¹

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Cellulose undoubtedly exerts some influence on the fertility of soils, since it is the major plant constituent entering the soil. The exact fate of cellulose in soil is not known, although cellulose is observed to decompose rapidly when in contact with moist soils.

Many experiments have been conducted in an attempt to determine the nature of cellulose decomposition as it would occur under natural circumstances, but few have been particularly enlightening, principally because of incomplete knowledge regarding the characteristics of the agents causing cellulose decomposition, the aerobic cellulose-decomposing organisms, and because most of the studies have been carried out on unnatural substrates, such as filter paper. Cellulose in plants is usually associated with other constituents that must exert some influence on its decomposition. Cellulose is not the same in all plants or even in the same plant at different stages of maturity.

The purpose of this investigation was to study the decomposition of cellulose, both alone and in the presence of other plant constituents, by pure cultures. In order to fulfill this purpose the following objectives were carried out: (1) Cellulose dextrans were prepared and used for the isolation of some aerobic mesophilic cellulose-decomposing organisms from the soil. (2) The characteristics of the cellulose-decomposing organisms were examined. (3) The decomposing ability of the organisms was studied on filter paper, cornstalk cellulose, and jute cellulose. (4) The influence of the presence of cellulosan (principally xylan), polyuronide hemicelluloses, and lignin on the extent of cellulose decomposition was investigated by subjecting various preparations of cornstalk cellulose and jute fiber to the activity of aerobic cellulose-attacking bacteria.

The isolation of some aerobic mesophilic cellulose-decomposing bacteria was accomplished by the use of water-insoluble cellulose dextrin agar medium. The production of a halo formation around restricted colonies on the opalescent medium served positively to identify active cellulose-decomposing bacteria, including the less versatile cytophagas.

Water-insoluble cellulose dextrans, ranging in average chain length from 75 to 25 anhydroglucose units, were prepared by hydrolyzing cellulose in cold 72 per cent sulfuric acid for $\frac{1}{2}$ to 5 hours, respectively. No one dextrin preparation in agar medium was found to have an advantage over the others for plating cellulose-decomposing organisms.

Suspensions of Clarion and Fayette silt loam of various dilutions

¹ Original thesis submitted August 17, 1942. Doctoral thesis number 681A.

were plated, using dextrin agar media. The results indicated that the dextrans are useful for enumerating cellulose-decomposing organisms of the soil. The most desirable concentration of the dextrin in agar media was 0.1 per cent.

The cultures of aerobic mesophilic bacteria that were characterized include three species of cytophaga similar to the original *Spirochaeta cytophaga*, five cultures belonging to the genus *Cellulomonas*, and one culture classed in the order of *Myxobacterales*. Except for the cytophagas and one culture of the genus *Cellulomonas*, the different organisms were capable of utilizing a wide variety of carbon sources, including the simple sugars, glucose, xylose, maltose, galactose, and the more complex substances, starch, cellulose, hemicellulose, pectin, and calcium gluconate. All cultures used yeast water extract and peptone as well as ammonia and nitrate as sources of nitrogen.

All the cultures decomposed the isolated plant cellulose far more extensively than extracted cellulose low in xylan or filter paper. This was particularly the case when the preparations were subjected to attack by those cultures that could use the extracted cellulose or filter paper only to a limited extent. The decomposition of the isolated cellulose by the pure cultures decreased in the following order: cornstalk cellulose (28 per cent xylan) > jute cellulose (15 per cent xylan) = extracted cornstalk cellulose (12 per cent xylan) > extracted cornstalk cellulose (7 per cent xylan) = filter paper (no xylan).

The portion of the xylan associated with cellulose that was most easily extracted by alkali was preferentially decomposed by all cultures and promoted the attack on the true cellulose, whereas the portion of xylan that resisted removal by prolonged acid and alkali treatments also resisted biological attack and was removed only concurrently with the cellulose. This indicates that the influence of the xylan on cellulose decomposition is as much a matter of the nature of the xylan as the amount.

The similarity between the hydrolysis of xylan in cellulose by dilute acids and the enzymatic action of bacteria was pointed out. The explanation for the resistance of a small portion of xylan to both acid hydrolysis and biological activity may be the same. All xylan does not appear to have the same chain length. If this is the case, it would be logical to suspect that the xylan having the shortest chain length would be both the most easily hydrolyzable by acids and the most available to biological attack. Another possibility for the resistance of a small portion of the xylan in cellulose is that mixed chains of xylose and glucose may occur, arranged in such a fashion that a chain of anhydroxylose units is connected to a chain of anhydroglucose units. The portion of the xylan so constituted in long chains with glucose would be likely to resist extraction by alkali and hydrolysis by acid solution, as well as resist biological attack. The anhydroxylose units would be oriented along with anhydroglucose units, and one could be removed only concurrently with the other.

The preferential attack on about 70 per cent of the xylan in cornstalk cellulose, together with the fact that, when alone, the decomposition of

true cellulose is difficult to initiate unless large mass inoculations are used, indicate the initial attack on cellulose may not be energy-yielding, thus supporting the theory that this attack is hydrolytic and not oxidative. In addition, these findings indicate that the exoenzyme system is rather restricted and becomes active only when the organisms are in close proximity to the fiber. The last point is also supported by the fact that at no time has the decomposition of cellulose been shown to occur in the absence of the organism by press juice. These studies clearly demonstrate that the mechanical and physical properties of the plant material and the architectural arrangement of the plant constituents in the natural fiber are of utmost importance in decomposition.

Lignin retards the decomposition of cellulose, especially when present in large amounts. The amount of lignin in plant material necessary to retard cellulose decomposition cannot be fixed at a definite value since lignin is not homogeneous, but varies with plants of different species and even with the same plant at different stages of maturity. The decomposition of the cellulose in the jute preparations by the most active cultures was only seriously decreased when the lignin content was about 6 per cent or greater. On the other hand, the attack on cellulose by the less active cultures was only noticeably decreased when the lignin content of the jute preparations was 12 per cent.

If the theory that lignin aids in the conservation of nitrogen in the soil by immobilizing protein and ammonium and retarding cellulose decomposition is correct, the amount of lignin in the plant materials sufficient to retard cellulose decomposition has practical applications that deserve attention.

IDENTIFICATION OF CLAY MINERALS IN SOME IOWA AND NEW ENGLAND SOIL PROFILES¹

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The chemical constitution and thermal curves of five common clay minerals were studied by Le Chatelier (3) as early as 1887. However, it was not until 1930, when Hendricks and Fry (2) demonstrated that soil-mineral colloidal material consisted largely of clay minerals, that widespread interest developed in the identification and characterization of clay minerals and soil colloids. X-ray and optical studies and complete chemical analyses have been the methods most frequently used in the identification of pure clay minerals. These procedures are of much less value in the identification of clay minerals as they occur in agricultural soils, intermixed with other clay minerals, hydrous oxides of iron, amorphous materials, and organic matter. It has been shown that certain indirect methods may be useful in identifying clay minerals, alone or in mixtures.

This investigation was an attempt to apply several indirect methods of clay mineral identification to the colloidal clay fraction of five Iowa and five New England soil types. In order to make studies of the thermal reactions of soil clays it was necessary to construct apparatus suitable for this work. Satisfactory equipment was assembled, patterned after that used by Norton (4).

Studies were conducted on soil colloids extracted from the following Iowa soils: four horizons in Grundy silt loam, three in Weller silt loam, three in Clarion loam, and one each in Webster silty clay loam and Tama silt loam. An attempt was made in this selection to obtain soil types which had developed from different parent materials and under several different vegetative covers. Curves obtained by the application of the thermal method to soil colloids extracted from these soils gave unmistakable evidence that montmorillonite was the predominating clay mineral in Iowa soils. Since it was not possible to identify illite by the thermal method in clay mineral mixtures containing montmorillonite and kaolinite, the suggestion of Alexander, Hendricks, and Nelson (1) was followed in identifying this mineral. Chemical determinations were made of the nonexchangeable potash content of soil colloids and the illite content calculated on the assumptions that (A) all nonexchangeable potash is contained in illite, and (B) illite contains on the average 4 per cent potash.

The percentage composition of the various minerals in the soil colloid were estimated from the potash content and the relative sizes of their endothermic reactions when compared with similar endothermic reac-

¹ Original thesis submitted October 31, 1942. Doctoral thesis number 688A.

tions obtained from pure minerals. Actual base-exchange capacity determinations were obtained for each soil colloid sample. These actual values were compared with calculated values based on the assumption that montmorillonite had a base-exchange capacity of 100, illite 30, and kaolinite 10 M.E./100 grams of colloid. The agreement between the two sets of values was good. There were only two comparisons which varied more than 6 M.E./100 grams of colloid.

Studies were conducted on soil colloids extracted from the following New England soils: two horizons in Hadley very fine sandy loam, three in Agawam fine sandy loam, five in Merrimac fine sandy loam, three in Paxton fine sandy loam, and three in Gloucester fine sandy loam. Thermal curves obtained from the colloids of these soils indicated the presence of considerable illite. This suggestion was confirmed by chemical analyses which revealed the presence of nonexchangeable potash averaging approximately 2.25 per cent potash. Since these soil colloids contain on the average more than 60 per cent illite, it is expected that they will react in the same way as this clay mineral. A comparison of the actual base-exchange capacities of these soil colloids with calculated exchange capacities revealed close agreement except when extracted surface soil colloids were compared. A pronounced characteristic of the New England soil colloids was the presence of two minor minerals, goethite and gibbsite. Neither of these was observed in the colloids extracted from Iowa soils. It was found necessary to remove free iron from some of the New England soil colloids before kaolinite could be identified by thermal analysis. Apparently some hydrous oxides of iron give exothermic reactions over the temperature range where kaolinite reacts endothermically. It appears necessary to obtain thermal curves on soil colloids similar to those found in New England both before and after the removal of free iron.

Data were obtained on the hydration and dehydration of pure minerals and soil colloids extracted from selected soil samples from New England and Iowa. These data tend to support the conclusions drawn from data on thermal and chemical analyses.

A brief preliminary study was conducted on the effect of soil particle size on the thermal curve. This study indicated that particles smaller than 2 microns may be satisfactorily used for thermal analysis. It is not known how well thermal curves may be related to chemical analyses and hydration studies on soil particles of this size.

SUMMARY

1. No single method is available which gives satisfactory results in the identification and quantitative estimation of clay minerals in soil colloids.

2. The most satisfactory quantitative estimation of clay minerals in soils was made by integrating data from thermal analyses, base-exchange capacity, and nonexchangeable potassium determinations.

3. Satisfactory thermal curves were obtained with simple apparatus constructed from standard laboratory equipment.

4. Colloids from the five Iowa soils are typified by the mineral montmorillonite. These soil colloids contain approximately 60 per cent of this mineral, in addition to 30 per cent illite and 10 per cent kaolinite.

5. Colloids from the five New England soils show considerable variability but are best characterized by illite. These soil colloids contain approximately 55 per cent illite, 20 per cent montmorillonite, 5 per cent kaolinite, 5 per cent gibbsite, and 2 per cent goethite.

6. Thermal curves should be determined on soil colloids similar to those from New England both before and after the removal of free iron. Treatment for the removal of free iron is apparently unnecessary for soil colloids similar to those from Iowa soils.

7. Pretreatment methods used to remove organic matter and free iron markedly affect the thermal curve and base exchange values.

8. Satisfactory thermal curves can be obtained on soil samples composed of particles smaller than 2 microns in diameter.

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THE EFFECT OF WORK UPON THE NITROGEN, CALCIUM, AND PHOSPHORUS BALANCES OF DRAFT GELDINGS¹

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The primary purpose of the study was to determine the effect of work upon the nitrogen balances of draft horses. However, the experimental plan made it possible simultaneously to obtain data on the effect of work upon the calcium and phosphorus balances of horses.

Data were collected in a metabolism experiment on two Percheron geldings, 4 and 5 years old, that weighed 1,674 and 1,681 pounds at the beginning of the trial. The horses were fed a basal daily ration composed of 3 pounds of finely ground oats and 20 pounds of finely chopped hay throughout the experiment. During the work periods the gross energy of this basal ration was increased by adding sugar and dextrinized corn starch as the situation demanded.

The experiment was of 11 weeks duration—2 weeks preliminary maintenance, 8 weeks metabolism, and one week post-work maintenance. The metabolism period consisted of maintenance, light, medium, and hard work periods, each of 2 weeks duration.

The collection of excreta was accomplished by the use of especially made metabolism stalls and harnesses. Aliquot samples of both urine and feces were taken each day for compositing into weekly samples, which were preserved and analyzed subsequently for nitrogen, calcium, and phosphorus.

Through the use of a dynamometer, designed and constructed by the Iowa State College Agricultural Engineering Department especially for this experiment, the amount of work performed by each gelding was quite readily controlled. During the light, medium, and hard work periods the dynamometer was set at 100, 170, and 240 pounds tractive pull, respectively. Accordingly, the horses developed 0.56, 0.93, and 1.27 horsepower during the respective work periods.

During the preliminary and experimental maintenance periods, the daily ration of 3 pounds of oats and 20 pounds of timothy hay was adequate to maintain the weights of both horses. On the other hand, it was difficult to maintain their weights during the work periods which followed, because, in spite of the use of sugar, the dextrinized corn starch could not be made palatable enough to induce the horses to eat it in sufficient amounts without running the risk of frequently throwing them "off-feed." However, the losses were not large. Bob lost only 86.9 and Mike 79.1 pounds during the 6 weeks of work. The digestibility of the ration appeared to decrease as the amount of work increased to a certain point, beyond which no appreciable change was observed.

¹ Original thesis submitted December 17, 1941. Doctoral thesis number 669.

The fact that the amount of food nitrogen consumed was always greater than the combined output in the feces and urine indicates strongly that the daily maintenance ration of 3 pounds of oats and 20 pounds of timothy hay provided sufficient protein during rest to meet the nitrogen requirements of the geldings. This was equally true during work, even though the additions of sugar and dextrinized corn starch to the basal ration widened the nutritive ratio from 1:10.2 to 1:16.8, 1:21.0, and 1:21.9 during the light, medium, and hard work periods, respectively. When considered on the basis of nitrogen retained, there is some doubt as to the wisdom of narrowing the nutritive ratio of horses simply because harder work is being required of them. On the contrary, rations having wider nutritive ratios may prove to be as efficient and often more economical.

Both horses were in negative calcium balance throughout the trial, which was undoubtedly due to the comparatively low percentage of calcium in each of the feeds fed. The timothy hay, the oats, and the corn starch analyzed 0.19 per cent, 0.06 per cent, and a trace of calcium, respectively. The intakes of calcium were quite uniform, but the outputs varied somewhat. Considering the whole trial, Bob retained more of the calcium he consumed than Mike. The latter excreted 51 grams more calcium than the former while consuming practically the same amount of calcium and doing the same amount of work.

The situation with reference to the phosphorus balance is somewhat similar to that of the calcium balance in that the feeds fed were abnormally low in phosphorus, and the phosphorus intake was hence insufficient to maintain a positive phosphorus balance. The percentage of phosphorus in the oats was 0.33 per cent, in the timothy hay 0.15 per cent, and in the corn starch 0.01 per cent. The total intake of phosphorus for the trial per horse was only 34 grams larger than the total intake of calcium. Mike's total output for the whole trial was 355 grams greater than Bob's.

The average amounts of calcium and phosphorus consumed daily by each horse during the maintenance period were 18 grams and 18.29 grams, respectively; the calcium to phosphorus ratio being 1:1.02. The maximum average amounts of calcium and phosphorus were consumed by Bob in the second week of hard work when his daily intake of calcium was 18 grams and his daily intake of phosphorus was 18.86 grams. The calcium to phosphorus ratio was 1:1.05.

Neither the maintenance ration nor the ration fed the horses at work supplied sufficient amounts of calcium and phosphorus to meet their requirements. On this basis, it appears probable when horses are fed rations as low in calcium and phosphorus as were fed in this experiment that many of them will receive insufficient calcium and phosphorus to satisfy their physiological and nutritional needs.

During this experiment in which the total outputs of calcium and phosphorus always exceeded the total intakes, various amounts of work did not seem to affect significantly either the calcium or phosphorus balances of the horses.

In this trial where the horses were in positive nitrogen balance and in negative calcium and phosphorus balances, the horses behaved more nearly alike with reference to nitrogen and calcium retention, but behaved differently with reference to phosphorus retention. The mean nitrogen retention ($1,417 \pm 16$ grams, 1 degree of freedom) and the mean calcium retention (-751.5 ± 12.5 grams, 1 degree of freedom) were significantly different from zero. The mean phosphorus retention (-687 ± 186 grams, 1 degree of freedom) was not significantly different from zero. The data were further analyzed using analysis of variance. In no cases were significant differences found that were due either to the level of work or the reaction of the horses to the level of work.

SOLVENTS IN ORGANOMETALLIC CHEMISTRY¹

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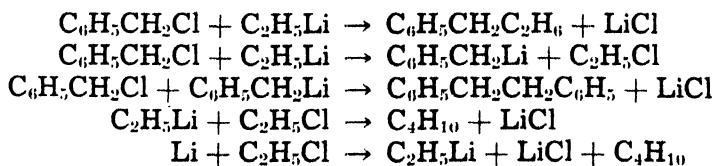
A search of the literature for solvents in organometallic chemistry reveals that ether is of predominate importance as a solvent in organometallic chemistry. Unfortunately, ether suffers the disadvantage of being cleaved by the more active organometallic compounds. This cleavage reaction is of such importance with ether solutions of organolithium compounds that a thorough study seemed necessary.

In order to undertake such a cleavage study a simple accurate method of analysis of solutions of organolithium compounds was needed. A method was devised which consisted of hydrolyzing an aliquot and titrating the total base formed. A second aliquot was added to an ether solution of benzyl chloride. The organolithium gave immediate and quantitative coupling with this organic halide to split out lithium chloride. The solution was hydrolyzed and titrated as before. The base formed in this hydrolysis was only the inorganic base such as LiOH, Li₂O, and ROLi so that the difference between the first and second titration gave the organolithium content of the solution. The analysis gave excellent results with all organolithium compounds except methyl-, phenylethynyl-, and aryllithium compounds.

When benzyl chloride reacts with *n*-butyllithium, bibenzyl, amylbenzene, and octane are found as the products. Since no other products could be isolated it was decided that a free-radical mechanism could not explain this reaction. When alkyllithium compounds were added to the benzyl chloride dissolved in ethyl ether, a yellow color flashed through the solution. Benzyllithium, a yellow compound, was thought to be formed as a result of this observation according to the following coupling equation.



When the coupling reaction was run at -50° the halogen-metal interconversion predominated so that the yellow color was maintained for sufficient time to carry out a carbonation reaction with dry ice. A small amount of phenylacetic acid was isolated and characterized as the *p*-bromophenacyl ester. The products of this coupling reaction may be formed as a result of the following equations.



¹ Original thesis submitted July 14, 1942. Doctoral thesis number 678A.

No bibenzyl could be isolated from the reactions with aryllithium compounds and benzyl chloride. This adds additional support to the above mechanism of the coupling reaction because no halogen-metal inter-conversion reactions are known between aryllithium compounds and alkyl halides.² Likewise, methyllithium, which is too unreactive to undergo halogen-metal interconversion, does not couple with benzyl chloride.

This mechanism may be applied to other abnormal coupling reactions between organolithium compounds and organic halides. It is not unreasonable to assume that this mechanism might explain the same type of reaction found in the coupling of certain Grignard reagents with organic halides.

The cleavage of ethers by organolithium compounds was studied by removing an aliquot from time to time and analyzing for the organolithium content of the solution by the benzyl chloride analysis. So long as there is an excess of ether the presence of an inert solvent has no effect upon the rate of cleavage.

With the exception of isopropyl-, cyclohexyl-, *s*-, and *t*-butyllithium the cleavage reaction in the presence of excess ether is one of the first order, and a rate constant may be calculated. With the above mentioned compounds, however, there is a rapid initial cleavage phase followed by a slow cleavage phase.

The organolithium compound which is the most stable in diethyl ether is methyllithium. 0.54 *N* solution of this compound was found to be 0.14 *N* after 1 year. *t*-Butyllithium was found to cleave diethyl ether the most rapidly, a 0.14 *N* solution decomposing in 30 minutes at room temperature.

Temperature has a marked effect on the rate of cleavage of ether. At room temperature (25°) 18 days were required to cleave a 0.65 *N* solution of *n*-butyllithium, while at reflux temperature, only 10° higher, a solution of the same concentration required only 5½ days for complete decomposition.

The aromatic organolithium compounds cleave ethers less readily than the alkylithium compounds. While a 0.4 *N* solution of *n*-amyllithium is decomposed by refluxing in ether for only 4 days, a 0.4 *N* solution of phenyllithium when refluxed for 30 days was found to be 0.09 *N*.

The order of increasing stability of organolithium compounds in diethyl ether at reflux temperature was found to be *t*-butyl < *s*-butyl = isopropyl = cyclohexyl < isobutyl < dodecyl = *n*-propyl < ethyl < *n*-butyl < *n*-amyl < *α*-naphthyl < phenyl < *p*-dimethylaminophenyl < *p*-biphenyl < methyl. This follows roughly the order of decreasing ability of these compounds to metalate or to effect halogen-metal interconversion.³

The stability of simple ethers (R₂O) in the presence of *n*-butyllithium has been found to increase in the order dodecyl < ethyl < isopropyl < *n*-butyl < *n*-hexyl. In the presence of *s*-butyllithium the order of in-

² Gilman and Jones, *Jour. Am. Chem. Soc.*, 63, 1441 (1941).

³ Gilman, Moore, and Baine, *Jour. Am. Chem. Soc.*, 63, 2479 (1941).

creasing stability was ethyl < *n*-hexyl < *n*-butyl, while *t*-butyllithium was more stable in butyl ether than in diethyl ether.

Although dialkylaminomethyl alkyl ethers are cleaved by Grignard reagents,¹ organolithium compounds are superior for this reaction in that the reaction is more rapid, the yields are greater, and a larger variety of compounds can be formed. This ether cleavage reaction gives a means of introducing the R_2NCH_2- group into a large number of molecules. The diethylaminomethyl group was introduced in this manner in place of the lithium atom in *p*-dimethylaminophenyllithium, in 4-lithiodibenzofuran, prepared by metalation of dibenzofuran, and in 5-ethyl-2-lithiocarbazole, prepared by the halogen-metal interconversion of 5-ethyl-2-bromocarbazole. Unfortunately, the addition of organolithium compounds to the anil linkage goes more rapidly than the cleavage of the R_2NCH_2OR compounds so that this reaction could not be carried out with such compounds as 3-quinollythium.

Halogen-metal interconversion between phenylethynyl chloride and *n*-butyllithium was found to take place. The interconversion of vinyl bromide was unsuccessful due to the splitting out of HBr with subsequent metalation of the acetylene formed to give acetylenedicarboxylic acid on carbonation. *o*-Bromophenol may be made to undergo interconversion with ethylmagnesium bromide in the absence of solvent and at high temperature. Although no interconversion took place with this halide and ethylaluminum iodides under these conditions, the reaction took place if 4-iododibenzofuran was employed.

Ethylmagnesium bromide was found to metalate dibenzofuran in the absence of solvent and at high temperature. Under these conditions no metalation occurred with triethylaluminum. Ethylaluminum iodides were unsuccessful as metalation agents for dibenzofuran since a Friedel-Crafts reaction took place on carbonation to produce 2-dibenzofurancarboxylic acid.

¹ Robinson and Robinson, *Jour. Chem. Soc.*, 123, 542 (1923).

A DETERMINATION OF THE ELASTIC CONSTANTS OF BETA-QUARTZ¹

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The experimental techniques available for evaluating elastic properties of anisotropic or isotropic solids can be divided into two general classes. The static method consists in observing the deformation of loaded bars or plates. The constants measured in this way are termed isothermal since in the process the small temperature changes caused by flexing the specimen have ample opportunity to become equalized. In the dynamic method, on the other hand, the elastic properties are inferred from the speed of sound in the medium. Standing waves are set up between opposite faces of the specimen. Knowing the frequency, dimensions, density, and mode of vibration, the elastic constants can be calculated. In this case small temperature differences will exist between regions of nodes and loops. The constants will differ slightly from those computed by the static method and are termed adiabatic.

The advantages of utilizing high order harmonics in calculations of adiabatic elastic constants from vibrating plates were first pointed out by Atanasoff and Hart (1), (2). They have clearly demonstrated that as the number of nodal planes between two opposite surfaces of a vibrating plate are increased, the perturbing effect of the edges has a diminishing influence on the frequency " f/n ," where " f " is the observed frequency of the harmonic and " n " the order of the harmonic. Under these conditions the specimen can safely be regarded as a good approximation to a plate with infinite lateral dimensions. The frequency " f/n " then depends only on the thickness of the plate, the mode of vibration, and the elastic properties of the medium. The theoretical treatment of the present subject is the same as that used by Atanasoff and Hart in their work on alpha quartz. An adjustment must be made however for the different symmetry conditions encountered in beta-quartz which is classified as D_6 , while alpha quartz is D_3 .

The announcement of Osterberg and Cookson (3) that elastic vibrations of considerable vigor could be maintained in beta-quartz by means of its piezoelectric effect suggested the feasibility of determining the elastic constants for this substance by dynamic methods. To induce vibrations in a parallelepiped of a piezoelectric substance, the specimen is placed between plane parallel electrodes to which is applied an alternating voltage of the proper frequency. Since the specimen between the electrodes must be at a temperature above 573°C if observations are to be made on beta-quartz, a special holder was devised. Nickel electrodes were used with iron rods as leads to the exterior of the furnace. A sheet of mica placed

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over the face of each electrode prevents contact with the specimen and reduces likelihood of breaking the plate due to nonuniform conduction of heat from the electrode. Unglazed porcelain plates and cylinders make up the balance of structural material. The holder assembly fits snugly in a resistance furnace heated by direct current. A chromel-alumel thermocouple was used to measure the temperature of the holder adjacent to the specimen.

The alternating voltage which is applied to the electrodes is obtained from a high frequency vacuum tube oscillator. This oscillator is frequency modulated by a 60 cps tone, causing the carrier frequency to sweep periodically across a band of controlled deviation. If a resonance frequency of the crystal lies within the range of maximum and minimum values of frequency attained by this modulated carrier, the block will absorb some energy from the field at a certain instant and start to vibrate. However, the frequency of the applied field is continually changing and, hence, remains only for an instant at the value it had when the crystal resonated. The quartz, having very low damping, will continue to vibrate for some little time, even though it receives no more energy from the applied electric field.

Thus, for a short time, two radio frequencies are present in the circuit; the first, a continually changing one of constant amplitude due to the oscillator; the second, a fixed one due to the vibrating crystal whose amplitude is decreasing because of damping. These two frequencies are applied to a detector, and the result of the mixing and demodulation is a single varying-pitch audio beat note. This audio tone is amplified and applied to the vertical plates of a cathode ray tube whose sweep frequency is the same as that of the tone which modulated the carrier of the oscillator, in this case 60 cps. A tunable parallel resonant circuit is used between the crystal holder and detector. This unit is adjusted to resonate at the center of the frequency band covered by the modulated carrier and provides a convenient means for introducing a standard comparison frequency into the channel. To compare the frequency of the crystal resonant position on the fluorescent screen with a known standard, the signal from a standard oscillator is introduced by electro-magnetic coupling and a similar pattern obtained which is superposed on the screen with the crystal response. The standard oscillator is tuned until the central portions of the two patterns coincide. The standard oscillator then has the same frequency as the crystal harmonic being observed.

The coefficients in the determinantal equation which applies to a given orientation of the plate involve the density and thickness of the plate as beta-quartz in addition to the values for the resonant frequencies. Measurements of the density have been made by Day, Sosman, and Hostetter (4), and their results were used in the present calculations. The expansion coefficients for quartz determined by Jay (5) permit the thickness of the plates as beta-quartz to be calculated from the dimensions measured at room temperature. A sufficient number of plates were made with different orientations to yield a set of determinantal equations which could be

solved simultaneously for the unknown elastic constants they contained. The values obtained in this manner for the elastic constants of beta-quartz at 600°C. are the following: $C_{11} = 118.4 \times 10^{10}$, $C_{12} = 19.0 \times 10^{10}$, $C_{13} = 32.0 \times 10^{10}$, $C_{33} = 107.0 \times 10^{10}$, $C_{14} = 35.85 \times 10^{10}$ dynes/cm².

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THE DEVELOPMENT OF AN APPARATUS FOR PRODUCING A SALINE HYPOCHLORITE SOLUTION ELECTROLYTICALLY FOR ANTISEPTIC PURPOSES¹

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During World War I an antiseptic material developed by Doctors Dakin and Carrel, and called Dakin's solution, was found to be the most effective antiseptic for the treatment of war wounds.² After the war this antiseptic was introduced into civilian medicine and was found to be just as useful for the treatment of infections and commonplace wounds.³

Dakin's solution is a hypochlorite solution with a concentration of 0.45–0.50 grams of NaOCl per 100 cc. and is buffered to a pH of 8.5–10.2. Boric acid and sodium bicarbonate are the substances usually employed as buffers.

Dakin's introduction of a hypochlorite solution was not an innovation as hypochlorite solutions had been used as antiseptics since 1788. The use of earlier hypochlorite solutions was limited, however, because of their high alkalinity which caused wound irritation. Dakin eliminated this by the use of a buffer and introduced the first really successful hypochlorite antiseptic solution.

The main drawbacks to Dakin's solution are its instability and the difficulty connected with its preparation. The solution is very unstable and will retain its effectiveness when buffered only for a period of about 24 hours. Its preparation requires the services of an experienced chemist or pharmacist. As a result, Dakin's solution, requiring fresh preparation before use, has been replaced by other antiseptics which although often more expensive and less effective are more stable and can be kept on hand.

It is believed by many that if a low cost foolproof apparatus were developed which would enable an inexperienced person to prepare a hypochlorite solution of the same concentration as that prescribed by Dakin, hypochlorite solution would again come into prominence as an antiseptic.

Work was begun in 1929 at the Iowa State College on the development of such an apparatus. A cell designed in 1931³ was put on the market for a short while but was withdrawn in 1937. The work was continued and has culminated in the cells described in this paper.

Two different type cells were developed, the first, a batch type cell which could be retailed for about \$100.00 and the second, a continuous type which would cost about \$150.00.

The batch type cell consists of a flat, 1-inch platinum gauge cathode

¹ Original thesis submitted August 17, 1942. Doctoral thesis number 680A.

² *Abortive treatment of wounds*, Brit. Med. Jour. 2:609, 1915.

³ Sweeney, O. R., and Baker, J. E., *An electrolytic apparatus for the production of antiseptic sodium hypochlorite solution*. Iowa Eng. Exp. Sta., Bull. No. 111, 1933.

and a flat 1-inch rhodium gauge anode mounted in vertical planes about $1/32$ of an inch apart. In the experimental model these electrodes were sealed into pieces of 5 mm. pyrex tubing which were held apart by short pieces of the same size tubing. Mercury was introduced into the tubes and contact was made with the source of direct current by copper wires dipped into the mercury.

A Battery Booster type dry plate rectifier sold by a mail order house, with a capacity of 5–10 amperes at 6 volts, was used to supply the direct current.

A tentative design for a commercial model of this cell has been drawn up and can be seen in the original paper.

This type cell produces a hypochlorite solution of 0.475 grams NaOCl per 100 cc. with a pH of 9.3 at the rate of about 10 cc. per minute from a salt solution containing 25 grams NaCl and 1.5 grams NaHCO_3 per liter.

The cell is operated by introducing the salt solution into a beaker into which the electrodes are immersed. The current is turned on for a period of time depending upon the amount of solution desired. At the end of the period the solution is ready for use. In the commercial model a time clock has been introduced which shuts off the current and makes the operation of the cell foolproof.

The effect of initial temperature of the salt solution, the concentration of sodium chloride and of sodium bicarbonate were studied. It was found that a decrease in initial temperature decreased the hypochlorite concentration for a given time of electrolysis. An increase in the sodium chloride content increased the hypochlorite concentration while the reverse was true with the sodium bicarbonate.

It was found that this cell could be operated with an automobile battery as its source of current; however, the electrolysis proceeded at a lower rate than when the rectifier was used. The battery produced a solution much lower in temperature than did the rectifier. This type cell could easily be adapted for use in an ambulance.

The concentration of hypochlorite in the electrolyzed solution from this cell can be varied by varying the time of electrolysis.

A continuous type cell was developed with a $7/8$ -inch inverted cone-shaped perforated platinum iridium (80–20) sheet cathode and a rhodium anode of the same size and shape. These electrodes were placed in a horizontal position with the cathode above the anode at a distance of $1/16$ of an inch apart.

The same type of rectifier used for the batch cell was used with the continuous cell.

A special salt solution reservoir, delivery tube, and air inlet system were designed which permitted the salt solution to be fed directly into the cell. The flow of liquid through the cell was metered by an easily accessible outlet orifice. The rate of liquid flow was controlled by the height of liquid in the cell body which in turn was regulated by the air inlet system.

An automatic device was incorporated into the design of the cell

which turned the current on as soon as the valve from the reservoir was opened and which shut it off as soon as the flow of liquid from the reservoir ceased. Liquid from the reservoir could stop flowing either by closing the valve or as a result of its running dry.

The continuous cell produces a hypochlorite solution containing 0.482 grams of NaOCl per 100 cc. with a pH of 8.8 at the rate of 10.8 cc. per minute. The salt solution used contained 25 grams NaCl and 1.5 grams of NaHCO_3 per liter.

It was found that with both of these cells, decomposition of the electrodes with the formation of a sodium peroxide which imparted a bluish tint to the hypochlorite solution could be kept at a minimum by keeping the temperature within the cell below 54°C . Since the batch type cell ran at a lower temperature than the continuous cell, due to the cooling of the salt solution prior to electrolysis, there was no evidence of peroxide in the hypochlorite solution produced in that cell.

It was found that the solution from the batch type cell contained negligible amounts of chlorate (0.002-0.003 grams per 100 cc.) while there were no chlorates present in the solution from the continuous type cell. Both of these cells produce hypochlorite solution for less than 5 cents a liter.

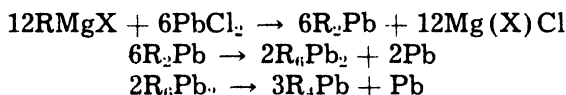
Both of these cells when properly constructed should have a long service life and should give little or no trouble during that time. They both draw around 5 amperes of current which is about half of the rated capacity of the rectifiers. This should lead to a long service life for the rectifier. The cells are simple to operate and could be handled by persons unskilled in chemical technique. The solution produced by them varies little in concentration and at all times falls within the limits prescribed by Dr. Dakin. The low price of these cells should enable them to be purchased by doctors and schools which could not afford more expensive equipment. These cells when put on the market should lead to a return of hypochlorite to the antiseptic field.

INTRODUCTION OF WATER-SOLUBILIZING GROUPS INTO SOME ORGANOMETALLIC COMPOUNDS¹

ROBERT W. LEEPER

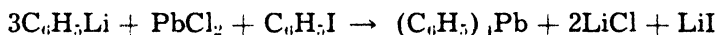
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Preparatory to a study of the introduction of water-solubilizing groups into organolead compounds, variations were made in the procedure for preparing tetraphenyllead. The following set of equations is usually given to explain the formation of an R_4Pb compound:



The reaction mixture is colored black by the free lead.

When phenyllithium was reacted with lead chloride in the presence of iodobenzene, tetraphenyllead was produced without the formation of free lead. The reaction can be written:



The yield of tetraphenyllead was 80 per cent based on this reaction or 160 per cent if based on the first set of three equations. If the iodobenzene was replaced by bromobenzene, the yield of tetraphenyllead was only 24.8 per cent. Under the same conditions phenyllithium and lead chloride alone produced 41 per cent of tetraphenyllead. Phenylmagnesium bromide, iodobenzene, and lead chloride produced 34 per cent of tetraphenyllead, and the reaction was colored black with free lead.

Hexaphenyldilead and maleic anhydride in chloroform, when allowed to stand for 5 months, produced α,β -di(triphenyllead)succinic anhydride. This was isolated as the free acid. An easier method of obtaining this product was to add hexaphenyldilead slowly to molten maleic anhydride and to extract the product progressively with benzene, alcohol, water, and ammonium acetate solution. The residue was insoluble in acetone, alcohol, dioxane, chloroform, carbon tetrachloride, benzene, and the petroleum ethers. It was slightly soluble in boiling water and dissolved in glacial acetic acid. It turned brown at 265-270° and had not melted at 330°.

The reaction between hexaphenyldilead and maleic anhydride was believed similar to that between triphenylmethyl and maleic anhydride.² Refluxing the free acid with 10 per cent sodium hydroxide slowly converted it to the sodium salt, as insoluble in water as the free acid.

Attempts to prepare ethyl α,β -di(triphenyllead)succinate from triphenyllead-sodium and ethyl α,β -dibromosuccinate in liquid ammonia were unsuccessful.

¹ Original thesis submitted December 9, 1942. Doctoral thesis number 696A.

² Conant and Chow, *Jour. Am. Chem. Soc.*, 55, 3475 (1933).

Triphenyllead acid maleate (m.p. 207°) was prepared in 90 per cent yield by boiling triphenyllead hydroxide and maleic anhydride in absolute alcohol. Di-triphenyllead maleate (sinters 198-199°) was prepared in 82 per cent yield in a similar way from two equivalents of the organolead compound in alcohol.

No reaction could be induced between hexaphenyldilead and benzene solutions of isoprene, pyrrole, styrene, furan, and 1,4-diphenylbutadiene-1,3, stored for 5 months.

Triphenyl-*o*-hydroxyphenyllead (m.p. 216-218°) was obtained in 12 per cent yield by adding *o*-bromophenol to a *n*-butyllithium solution in ether, stirring for ½ hour, adding an equal volume of benzene, followed by triphenyllead chloride. After refluxing for 1 hour the mixture was hydrolyzed with dilute hydrochloric acid and worked up in the usual manner.

9-Phenanthrylmagnesium bromide and triphenyllead bromide refluxed 24 hours in an ether-benzene solution gave a 57 per cent yield of triphenyl-9-phenanthryllead upon hydrolysis. Recrystallization from ethyl acetate gave a product melting at 169-171°.

In a similar manner diphenyllead dibromide gave a 68 per cent yield of di-9-phenanthryldiphenyllead (m.p. 208-210°). Hydrogen chloride cleaved the phenanthryl radicals.

Attempts to prepare tetra-9-phenanthryllead were unsuccessful.

If 7-bromo-1,2-benzanthracene was added to a *n*-butyllithium solution, followed by triphenyllead chloride 2 minutes later, hydrolysis gave a 52 per cent yield of triphenyl-7-(1,2-benzanthryl)lead. When recrystallized from ethyl acetate it melted at 204-205°.

In a similar manner diphenyllead dichloride gave a 3.6 per cent yield of di-7-(1,2-benzanthryl)diphenyllead (m.p. 295-296°). Hydrogen chloride cleaved the 1,2-benzanthryl radical.

The products from hexaphenyldilead and liquid ammonia solutions of lithium, sodium, potassium, rubidium, calcium, strontium, and barium were converted to triphenylbenzyllead with benzyl chloride. Highest yields were obtained with the first member of the group I-A and II-A metals (lithium 72 per cent, calcium 81 per cent), and the yields became progressively smaller as one went down the group. The group II-A metals formed products of the type $(C_6H_5)_3PbCaPb(C_6H_5)_3$ rather than $(C_6H_5)_3PbCaPb(C_6H_5)_3$.

Diphenyllead dichloride (0.01 mole) and calcium (0.01 g. atom) in liquid ammonia gave a 23.3 per cent yield of hexaphenyldilead and 10 per cent of tetraphenyllead. With diphenyllead difluoride the yield was 58 per cent of hexaphenyldilead. Diphenyllead dichloride (0.01 mole) and lithium (0.04 g. atom) gave 60 per cent of hexaphenyldilead. Diphenyllead difluoride (0.005 mole) and lithium (0.01 g. atom) gave a 52 per cent yield.

Hydrogen chloride bubbled into dicyclohexyldiphenyllead dissolved in petroleum ether (b.p. 28-30°) precipitated dicyclohexylphenyllead chloride (m.p. 195°; dec. 205°), soluble in chloroform. When a sus-

pension of this material (0.02 mole) in liquid ammonia was treated with sodium (0.02 g. atom), a deep red color developed. Chloroform extraction of the residue after the ammonia evaporated gave a deep red solution that was instantaneously decolorized in sunlight. It was impossible to isolate the expected *sym*-hexacyclohexyldiphenyldilead.

In a similar manner diphenylethyllead chloride (sinters 142°; dec. 146-147°) was obtained in 81 per cent yield from diethyldiphenyllead. It was impossible to isolate the expected *sym*-hexaphenyldiethyldilead when the chloride was treated with sodium in liquid ammonia.

Diphenyllead dinitrate³ was converted to di-*m*-nitrophenyllead dinitrate⁴ by nitration at -50°. When this was dissolved in boiling water and a sodium chloride solution added, di-*m*-nitrophenyllead dichloride precipitated in 97 per cent yield. It was soluble in alcohol and recrystallized in plates from ethyl acetate (sublimed 250°; dec. 285-289°).

In a similar manner a sodium iodide solution gave a 95 per cent yield of di-*m*-nitrophenyllead diiodide (dec. 135°).

Triethyllead chloride in benzene solution did not react with diazomethane or diazoethane.

Lead powder and ethyl α -bromopropionate, bromomethyl acetate, and ethyl bromoacetate did not react when heated.

When tetra-*n*-butylgermanium was warmed with iodine, tri-*n*-butylgermanium iodide (b.p., 126-128°) was obtained in 69 per cent yield.

2-Furyllithium and germanium tetrabromide refluxed 4 hours in benzene gave a 32 per cent yield of tetra-2-furylgermanium (b.p., 163°; m.p. 99-100°).

n-Butyltin triiodide (b.p., 154°) was obtained in 25 per cent yield from KSnCl_3 and *n*-butyl iodide heated 72 hours at 90° in a sealed tube⁵ (unstable).

Tin powder reacted with ethyl bromoacetate⁶, bromomethyl acetate, phenacyl bromide, β -bromoethyl acetate, ethyl α -bromopropionate, diethyl α -bromosuccinate, diethyl dibromomalonate, and 2-bromopyridine to give either glassy solids or thick tars. Only in the case of ethyl bromoacetate was it possible to isolate the pure product. The tar was washed out with ether, and the remaining solid was recrystallized from benzene to give a 15.5 per cent yield of dicarbethoxymethyltin dibromide (m.p. 139°).

Tin powder and 1-chloro-2-iodoethane, 1-bromo-2-chloroethane, and 1-bromo-3-chloropropane reacted to varying extents, but conditions for best reaction were also ideal for decomposition of the products.

³ Setzer, Leeper, and Gi'man, *Jour. Am. Chem. Soc.*, 61, 1609 (1939).

⁴ Challenger and Rothstein, *Jour. Chem. Soc.*, 1258 (1934).

⁵ Tchakirian, Lesbre, and Lewinsohn, *Compt. rend.*, 202, 138 (1936).

⁶ Compare with Emmert and Eller, *Ber.*, 44, 2328 (1911).

THE BIOLOGY AND MORPHOLOGY OF *COLASPIS FLAVIDA* (SAY)¹

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The recent outbreak of the grape colaspis, *Colaspis flavida* (Say), as a pest of corn in eastern and east central Iowa is discussed, and the results of the 1941 and 1942 survey activities are mapped. The current taxonomic status of the insect, according to Mr. H. S. Barber of the United States National Museum, is given, pointing out that little is known of the exact species which have been the subject of discussion by earlier authors, although all of them believed they were discussing the same insect. Therefore, the exact distribution of *C. flavida* cannot be ascertained at this time. Mr. Barber believes this species is probably limited to the upper Mississippi Valley and Great Plains area.

A rather complete review of all of the literature dealing with *C. flavida* complex is presented in chronological sequence, so that a history of its rise as an economic pest is the result. In addition, all the recorded host plants for the species-complex are given.

A detailed account of the life history of *C. flavida*, as it occurs in Iowa, is given. Egg laying is heaviest in July and August with hatching occurring within 1 or 2 weeks and with larval development up to approximately the sixth instar taking place before freezing weather sets in. A description of the hibernating larva is included so that it may be readily distinguished from one that is preparing to molt. The winter is passed in a quiescent state in the soil at a depth of about 10 inches, and it appears unlikely that surviving larvae have been above frost line. Development is resumed in the spring, and severe, though localized, injury may occur to corn planted on red clover ground. During the rearing experiments ten to seventeen larval instars occurred preceding pupation. Since the growth curve showed a constant rate of development in the instances of the ten instar individuals, ten instars are considered to be normal, with excessive molts supernumerary and probably due to adverse environment. Pupation lasts about 1 week, and adult emergence may begin late in June and continue into August, with the late emerging forms usually coming from dense growths of red clover or alfalfa where the ground had been well shaded. Adults may live from three to four weeks.

Sex ratios appear nearly equal, the female laying approximately 150 eggs, usually in two batches. The oviposition and feeding habits of the adults are discussed, with a record of extensive adult feeding upon *Polygonum lapathifolium*, a smartweed common in corn fields in eastern Iowa, included.

¹ Original thesis submitted May 17, 1943. Doctoral thesis number 715.

Various types of survey studies are discussed, and a recommendation for a proposed survey method is outlined. The application of such a survey method, as it affects the cultural practices of the farmer, is discussed. Under the section on control studies are included the apparently minor effects of parasites and predators. The effects of soil types are discussed and the correlation of the occurrence of *C. flavida* with the areas of light loess soils is pointed out. Effects of climatic conditions and cultural practices are also discussed.

Such morphological studies as were deemed helpful in determining the species from field collected specimens, of both larval and adult forms, are included. Methods of sexing both pupa and adults are discussed and illustrated. No definite differences in larval chaetotaxy were established. Descriptions of the representative forms from egg to adult are presented, and correlations of instars with head capsule widths are shown. Drawings showing the main points of morphological interest are included.

LONG-CHAINED ORGANOMETALLIC COMPOUNDS¹

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Relatively few long-chained organometallic compounds have been prepared; aside from a few mercury, magnesium, and lithium derivatives the subject has in the past received very little attention. The hope that these high molecular weight compounds might by reason of their solubility, low vapor pressure, and relative noninflammability find uses from which the lower homologs were barred, led to this investigation.

Solutions of long-chained alkyl bromides and chlorides in ether reacted with lithium readily. The bromides yielded less than 50 per cent of lithium alkyls, but the chlorides gave 75 per cent yields. The solutions were titrated by the benzyl chloride double titration method.² The yields as measured by simple acid titration³ were 65 per cent and 100 per cent, respectively, for the bromides and chlorides.

In low-boiling petroleum ether (30-35°) the long-chained chlorides reacted slowly with lithium, and yielded about 50 per cent of the lithium alkyls; the bromides gave very low yields. This is in contrast to the work of Moore,⁴ who found that the lower homologs yielded 85 per cent and 60 per cent, respectively, with chlorides and bromides. Moore's results were based upon simple acid titrations, which give values considerably too high even in petroleum ether.

In ordinary petroleum ether (60-68°) or in benzene, long-chained chlorides reacted readily with lithium at reflux temperature, yielding from 57 to 74 per cent of organolithium compounds. The bromides gave about 15 per cent yields under the same conditions. Gilman, Langham, and Moore⁵ found this petroleum ether fraction to be less satisfactory than the low-boiling fraction, with the lower alkyl halides.

Carbonation of the long-chained lithium alkyls by pouring into dry ice and ether resulted in the formation of ketones as well as acids, and since the ketones could not be separated quantitatively from the coupling products, carbonation was not suitable as a means of checking the titration yields. Small amounts of dodecane, dodecene, and tetra-cosane were found among the products of reaction between dodecyl chloride and lithium.

Long-chained alkylsodium compounds were prepared from the alkyl chlorides and sodium metal. Petroleum ether (30-35°) was a suitable solvent. The long-chained sodium alkyls were not soluble in any of the

¹ Original thesis submitted July 13, 1942. Doctoral thesis number 674A.

² A. H. Haubein, unpublished work.

³ Gilman, Wilkinson, Fishel, and Meyers, *Jour. Am. Chem. Soc.*, 45, 150 (1923).

⁴ F. W. Moore, Doctoral Thesis, Iowa State College (1941).

⁵ Gilman, Langham, and Moore, *Jour. Am. Chem. Soc.*, 62, 2327 (1940).

solvents used—petroleum ether, benzene, kerosene, and ether. The yields were estimated by carbonation with dry ice.⁶

n-Dodecyl chloride and sodium reacted in petroleum ether at 15-20° to give, generally about 15 to 20 per cent of dodecylsodium (though in the presence of a large excess of sodium⁷ the yield of dodecylsodium was as high as 42 per cent) 15 to 20 per cent of dodecene, 20 to 30 per cent of dodecane, and about 20 per cent of tetracosane.

When the preparation was carried out in benzene at room temperature, benzoic acid was one of the products obtained on carbonation, indicating that the long-chained sodium alkyls metalated benzene under mild conditions. The yield of benzoic acid decreased with increasing length of the alkyl chain.

In diethyl ether dodecyl chloride gave, after 2 hours at 0°, about 1 per cent of dodecylsodium, which yielded tridecylic acid on carbonation. The yields of dodecene, dodecane, and tetracosane were 15 per cent, 56 per cent, and 10 per cent, respectively; the high ratio of dodecane to dodecene was probably due to cleavage of the ether.

Carbonation of dodecylsodium with gaseous carbon dioxide resulted in the formation of small amounts (1.5 per cent) of undecylmalonic acid, in addition to the tridecylic acid.

Potassium metal reacted with dodecyl chloride; after carbonation of the resulting mixture there was obtained 10 per cent of the theoretical yield of tridecylic acid, 37 per cent of dodecane, 16 per cent of dodecene, and 24 per cent of tetracosane.

Long-chained alkyl bromides gave about 75 per cent yields of Grignard reagents; carefully purified samples gave as high as 87 per cent. Chain length, from dodecyl to octadecyl, had little effect on the results. The chlorides reacted much more slowly, but gave about 95 per cent of the organomagnesium compounds.

Hexadecyl iodide in ether reacted readily with fresh calcium filings. The products obtained after carbonation were about 13 per cent of margaric acid, 23 per cent of hexadecane and hexadecene, and 41 per cent of dotriacontane. Several attempts to prepare long-chained barium compounds were unsuccessful.

Didodecylmercury was prepared from dodecylmercuric bromide⁸ and dodecylmagnesium bromide. The other long-chained bismercurials were made analogously. Separation from the alkylmercuric halides was accomplished by crystallization from petroleum ether. The mercury dialkyls were crystalline solids, easily soluble in fat solvents, but only slightly soluble in alcohol and not at all in water.

Dodecylmercuric chloride was made by cleavage of didodecylmercury with mercuric chloride. The iodide, phosphate, and sulfate were prepared similarly. Silver acetate reacted with dodecylmercuric bromide

⁶ Gilman and Pacevitz, *Jour. Am. Chem. Soc.*, 62, 1301 (1940).

⁷ Morton and Richardson, *Jour. Am. Chem. Soc.*, 62, 123 (1940).

⁸ Hill, *Jour. Am. Chem. Soc.*, 50, 167 (1928).

to give dodecylmercuric acetate. Several of the other long-chained alkylmercuric halides were prepared by similar reactions.

Dodecylmagnesium chloride and lead chloride gave tridodecyllead chloride as the chief product. The other long-chained Grignard reagents reacted similarly. The reaction of trihexadecyllead chloride with hexadecylmagnesium bromide gave tetrahexadecyllead. This was a white crystalline solid, extremely soluble in petroleum ether, fairly soluble in ether or ethyl acetate, slightly soluble in alcohol, and insoluble in water.

Stannic chloride and a slight excess of dodecylmagnesium bromide yielded tetradodecyltin. The other long-chained tin tetraalkyls were obtained similarly. Tetrahexadecyltin resembled tetrahexadecyllead very closely. Tridodecyltin chloride was prepared by treating an ether solution of tetradodecyltin with dry hydrogen chloride at room temperature. It was less soluble in the fat solvents than tetradodecyltin, but more soluble than tridodecyllead chloride. The same relationships held for the higher trialkyltin chlorides.

Tridodecylarsenic and tritetradecylarsenic were prepared from arsenic tribromide and the Grignard reagents. Tridodecylarsenic was distilled without appreciable decomposition at 200° at 0.009 mm.; n_D^{35} 1.4740; d_{20}^{35} 0.900. Tritetradecylarsenic decomposed during distillation, but was obtained by the expedient of distilling out all the impurities in *vacuo*; n_D^{35} 1.4740; d_{20}^{35} 0.908.

TABLE 1
MELTING POINTS OF LONG-CHAINED ORGANOMETALLIC COMPOUNDS

Type of Compound	Alkyl Groups			
	<i>n</i> -Dodecyl	<i>n</i> -Tetradecyl	<i>n</i> -Hexadecyl	<i>n</i> -Octadecyl
R ₂ Hg.....	44-44.5°	53-54°	61-62°	66 5 67~
RHgCl.....	114-114.5		114 115	115 116
RHgBr.....	108-108.7	110-110.5	110 5-111.5	110 111
RHgl.....	91		93	
R ₄ Sn.....	15-16	33 34	41.5-42.5	47
R ₄ Pb.....		31	42	
R ₃ SnCl.....	33	46-47	55.5 56.5	61 62
R ₃ PbCl.....	64-65	74-75	79 80	82 83

The melting points of mixtures of related or homologous long-chained lead and tin compounds usually did not show depressions. Mixtures of the long-chained alkylmercuric halides showed small depressions of the melting point.

ORGANOLEAD COMPOUNDS CONTAINING WATER-SOLUBILIZING GROUPS¹

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The literature concerning studies of the preparation of organogermanium, organotin, and organolead compounds containing water-solubilizing groups has been reviewed.

Organolithium compounds are useful intermediates in the synthesis of unsymmetrical organolead compounds. The halogen-metal interconversion reaction² has been applied to the preparation of organolithium compounds from halides which react only to a slight extent or not at all with lithium metal to form organolithium compounds or with magnesium to form Grignard reagents. The halides which have been studied include (1) compounds containing unreactive halogen atoms, and (2) aromatic halides containing functional groups.

The following unreactive halides were converted to the corresponding organolithium compounds by interconversion with *n*-butyllithium in ether (the percentages given in parentheses after the names of the halides represent the yields of carboxylic acids obtained by carbonation of the organolithium compounds): 2,4,5-triphenyl-3-bromofuran (66 per cent); 2,4,5-triphenyl-3-chlorofuran (14 per cent); 3,4,6-triphenyl-2-bromopyridine at -35° (67 per cent); and 2-bromobenzofuran at -70° (62 per cent). The following new acids were prepared by this method and converted to their methyl esters by treatment with diazomethane: 2,4,5-triphenylfuran-3-carboxylic acid, m.p. $257-258^{\circ}$, methyl ester, m.p. $123.5-124^{\circ}$; 3,4,6-triphenylpyridine-2-carboxylic acid, m.p. $166-168^{\circ}$ (dec.), methyl ester, m.p. $117-118^{\circ}$.

The reaction of 3-bromobenzofuran with *n*-butyllithium was not a satisfactory means of preparing 3-benzofuryllithium. At room temperature the chief reaction was cleavage of the benzofuran ring to give *o*-ethinylphenol. Halogen-metal interconversion under most conditions, followed by carbonation of the reaction mixture, gave small yields of benzofuran-2-carboxylic acid.

Conditions for the preparation of *p*-bromobenzyl alcohol from *p*-bromotoluene in improved yields through the intermediates, *p*-bromobenzyl bromide and *p*-bromobenzyl acetate, have been described. The *o*- and *m*-bromobenzyl alcohols were similarly prepared. *p*-Bromophenethyl alcohol was prepared in 40 per cent yields by the reaction of *p*-bromophenylmagnesium bromide with ethylene oxide.

Halogen-metal interconversion was effected between *n*-butyllithium and some aromatic halides containing alcoholic hydroxyl groups. Two

¹ Original thesis submitted March, 1943. Doctoral thesis number 706.

² For general references on the halogen-metal interconversion reaction, see Gilman, *Organic Chemistry*, John Wiley & Sons, New York (1943), 2nd ed., vol. 1, p. 538.

moles of *n*-butyllithium were used, one to replace the active hydrogen atoms of the hydroxyl groups, and one to effect the interconversion. The best procedure was found to be addition of the *n*-butyllithium in ether to the bromo-alcohol. The minimal yields of interconversion products, as determined by carbonation and isolation of the resulting acids, were as follows: *p*-bromobenzyl alcohol (18 per cent), *m*-bromobenzyl alcohol (32 per cent), *p*-bromophenethyl alcohol (52 per cent), and *p*-bromo- α -methylbenzyl alcohol (45 per cent). Two of the acids obtained are reported for the first time: *p*-carboxyphenethyl alcohol, m.p. 127-128°, and *p*-carboxy- α -methylbenzyl alcohol, m.p. 138-139°. The melting point of *m*-carboxybenzyl alcohol, similarly prepared, was found to be 114.5-115°. The melting point previously reported³ for this compound is 111°.

p-Bromobenzenesulfonamide appeared to undergo halogen-metal interconversion to some extent when treated with two equivalents of *n*-butyllithium at room temperature, but pure *p*-carboxybenzenesulfonamide could not be isolated from the carbonated reaction mixture. *p*-Bromobenzonitrile has been shown to undergo interconversion to the extent of 10 per cent upon treatment with *n*-butyllithium in ether at -70°.

The synthesis of organolithium compounds by halogen-metal interconversion of nuclearily bromine-substituted phenylalkyl alcohols has provided intermediates for the synthesis of organolead compounds containing the alcoholic hydroxyl group. The reaction of the new organolithium compounds with triphenyllead chloride constitutes a general method for synthesizing triphenylhydroxyalkylphenyllead compounds. The reaction of triphenyllead chloride with the organolithium compounds formed by interconversion of *o*-, *m*-, and *p*-bromobenzyl alcohols, *p*-bromophenethyl alcohol, and *p*-bromo- α -methylbenzyl alcohol produced, respectively, the following organolead compounds containing alcoholic hydroxyl groups: triphenyl-*o*-hydroxymethylphenyllead, m.p. 134-136°; triphenyl-*m*-hydroxymethylphenyllead, m.p. 113-114°; triphenyl-*p*-hydroxymethylphenyllead, m.p. 98-100°; triphenyl-*p*- β -hydroxyethylphenyllead, m.p. 87-88°; and triphenyl-*p*- α -hydroxyethylphenyllead, m.p. 68-70°. The product of the reaction of triethyllead chloride with the organolithium compound from *p*-bromobenzyl alcohol decomposed on attempted distillation.

Triphenyl-*p*-hydroxymethylphenyllead was oxidized by potassium permanganate in acetone to give a 25 per cent yield of triphenyl-*p*-carboxyphenyllead, m.p. 256-258°. The sodium and potassium salts of this acid were insoluble in water. The free acid and diazomethane formed triphenyl-*p*-carbomethoxyphenyllead, m.p. 125-127°. The oxidation of triphenyl-*o*-hydroxymethylphenyllead with potassium permanganate produced the inner anhydride of diphenyl-*o*-carboxyphenyllead hydroxide, an amorphous compound which melted with turbidity at 300-305°. The formation of this substance resulted from oxidation of the hydroxymethyl group and simultaneous cleavage of a phenyl group to give diphenyl-*o*-carboxyphenyllead hydroxide which spontaneously lost water to give the anhydride. Treatment of the anhydride with hydrochloric acid in alcohol

³Langguth, *Ber.*, 38, 2063 (1905).

gave diphenyl-*o*-carboxyphenyllead chloride, melting at 210-220° with turbidity, and the latter with diazomethane formed diphenyl-*o*-carbo-methoxyphenyllead chloride, m.p. 170-171°. No pure product could be isolated from permanganate oxidation of triphenyl-*m*-hydroxymethylphenyllead.

Carbonation of the product of reaction of one mole of *p*-phenylenedilithium with one mole of triphenyllead chloride did not give the expected triphenyl-*p*-carboxyphenyllead. A small amount of *p*-phenylenedi-(triphenyllead), $(C_6H_5)_3PbC_6H_4Pb(C_6H_5)_3$, m.p. 285-288°, was formed. Triphenyl-*p*-carboxyphenyllead could not be prepared by the reaction of triphenyllead chloride with the product of halogen-metal interconversion of *p*-iodobenzoic acid. No reaction took place between triphenyllead chloride and sodium malonic ester. Triphenyl-*p*-cyanophenyllead could not be prepared by the reaction of cuprous cyanide with the diazonium salt obtained from triphenyl-*p*-aminophenyllead.

The reaction of *o*-anisylmagnesium bromide with triphenyllead chloride gave triphenyl-*o*-anisyllead, m.p. 128-129°. A similar reaction in the case of *p*-anisylmagnesium bromide gave triphenyl-*p*-anisyllead,⁴ which has previously been prepared from *p*-anisyllithium and triphenyllead chloride. The synthesis of triphenyl-*o*-methoxymethylphenyllead failed because only a small yield of the Grignard reagent could be obtained from methyl *o*-bromobenzyl ether.

Pure triethyl-*p*-bromophenyllead (d_{25}^{20} 1.8586; n_D^{20} 1.5968) was obtained by high-vacuum distillation (0.002 mm.; bath temp., 143°) of the product of the reaction of *p*-bromophenylmagnesium bromide and triethyllead chloride. The triethyl-*p*-bromophenyllead reacted with activated copper-magnesium alloy to give a Grignard reagent, but the acid formed upon carbonation could not be isolated in pure form.

The reaction of triethyllead-sodium with α -diethylaminopropyl chloride gave a product which could be distilled under high vacuum and which appeared to be impure triethyl- α -diethylaminopropyllead. An organolithium compound could not be prepared in good yield from α -diethylaminopropyl chloride and lithium.

The reaction of tetraphenyllead with nitrogen tetroxide in chloroform produced diphenyllead dinitrate. No pure products were isolated from the reaction of nitrogen tetroxide with triphenyl-*p*-hydroxymethylphenyllead or with triphenyl-*p*- β -hydroxyethylphenyllead.

A water-soluble product was formed by the reaction of tetraphenyllead with fuming sulfuric acid at 0°. Tetraphenyllead was cleaved by chlorosulfonic acid at low temperatures with the formation of diphenyllead dichloride.

Tetraphenyllead was recovered unchanged after having been refluxed for 3 hours with alcoholic sodium hydroxide. Both tetraphenyllead and triphenyl-*p*-anisyllead were recovered almost quantitatively after having been refluxed with chloroform and strong alkali under the conditions of the Reimer-Tiemann reaction.

⁴Towne, *Iowa State Coll. Jour. Sci.*, 8, 229 (1933).

REACTION OF GLUCOSE WITH SOME AMINES¹

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The purpose of this investigation was to study the reaction of glucose with a number of amines and to correlate the isolated investigations reported in the literature concerning certain amines and glucoses. Although a considerable amount of work has been done on the nitrogen derivatives of sugars, the literature regarding a great many of these derivatives is very confused. This is not surprising when it is realized that each of these derivatives may exist, theoretically, in a number of isomeric forms. The possibilities include addition compounds, N-glucosides of α - or β -furanoside or α - or β -pyranoside structure and the *syn* and *anti* Schiff base. Moreover, some of the N-glucosides rearrange to an isoglucosamine structure. The hydrolysis of the N-glucosides in aqueous solution and in the presence of dilute acids complicates the study further.

A few years ago Bergmann and Machemer (1) prepared the phenylhydrazine derivatives of the acetylated cellulose dextrins and reported that the nitrogen content of these derivatives was a better criterion for determination of molecular size than copper reducing values. Although the nitrogen content of phenylhydrazine derivatives of corn syrup dextrins prepared in this laboratory (2) gave a value of the molecular size comparable to that determined by iodine titration and copper-reducing values, the physical constants showed that the derivative was not homogeneous. Glucose and maltose derivatives of the amine were studied to obtain evidence regarding the structure of the dextrin derivative.

Phenylhydrazine and glucose reacted at room temperature to form crystalline, highly solvated compounds of varying composition. The solvation molecules could not be removed by ether extraction, but were removed by prolonged drying in a vacuum oven at temperatures between 50 and 75° C. The composition of the compound approached the monophenylhydrazine derivative, and the melting point approached 105-107° C. The ease of isomerization of the derivative was illustrated by formation of at least three modifications of the phenylhydrazine derivative by recrystallization of the α -hydrazide from alcohol. The ease of isomerization along with the tendency for formation of solvated molecules indicates that the identity of most of these isomers by chemical means is questionable but suggests that these derivatives are N-glucosides.

Several alkylamines were reacted with glucose under conditions that normally resulted in formation of an isoglucosamine from arylamines and glucose. In every case good yields of glucosyl-*n*-alkylamines were obtained. Among the compounds prepared in this manner were glucosyl-

¹ Original thesis submitted May 31, 1943. Doctoral thesis number 718.

n-butylamine [m.p. 96-97° C.; $[\alpha]_D^{25} = -22^\circ \rightarrow -7.8^\circ$ (c., 2 per cent in C_2H_5OH)], glucosyl-*n*-amylamine [m.p. 96-97°; $[\alpha]_D^{25} = -22^\circ \rightarrow -8^\circ$ (c., 2 per cent in C_2H_5OH)], glucosyl-*n*-heptylamine [m.p. 97-98°; $[\alpha]_D^{25} = -13^\circ \rightarrow -7^\circ$ (c., 2 per cent in C_2H_5OH)], and glucosyldicyclohexylamine [m.p. 97-98°; $[\alpha]_D^{25} = -23.5^\circ \rightarrow -11.6^\circ$ (c., 2 per cent in C_2H_5OH)]. By other methods glucosyl-*n*-octadecylamine (m.p. 104-105° C.), glucosyl-*n*-hexadecylamine (m.p. 106-107° C.), and diglucosylethylenediamine [m.p. 152-153° C.; $[\alpha]_D^{25} = -17^\circ \rightarrow +14.5^\circ$ (c., 2 per cent in 50 per cent C_2H_5OH)] were prepared. No crystalline compound was obtained from the condensation of glucose and 2-aminooctane. Propylenediamine and isopropylamine reacted with glucose to give compounds that were quite impure and from which no pure crystalline compound could be obtained.

The catalytic hydrogenation of the glucosylalkylamines was accomplished in a bomb with Raney nickel as a catalyst, at temperatures below 100° C. and 800 pounds or more of hydrogen pressure. The compounds isolated were *N*-butyl-*d*-glucamine [m.p. 126-127° C.; $[\alpha]_D^{25} = -14^\circ$ C. (c., 1 per cent in 50 per cent C_2H_5OH)], *N*-amyl-*d*-glucamine [m.p. 129-130° C.; $[\alpha]_D^{25} = -13.8^\circ$ (c., 1 per cent in 50 per cent C_2H_5OH)], *N*-heptyl-*d*-glucamine [m.p. 126-127° C.; $[\alpha]_D^{25} = -14^\circ$ (c., 1 per cent in 50 per cent C_2H_5OH)], *N*-cyclohexyl-*d*-glucamine [m.p. 145-146° C.; $[\alpha]_D^{25} = -11^\circ$ (c., 1 per cent in 50 per cent C_2H_5OH)], *N,N'*-ethylenediglucamine [m.p. (c., 136-137° C.; $[\alpha]_D^{25} = -15.5^\circ$ (c., 1 per cent in 50 per cent C_2H_5OH)], *N*-hexadecyl-*d*-glucamine (m.p. 123-124° C.), *N*-octadecyl-*d*-glucamine (m.p. 118-119° C.). Reduction of the glucosylisopropylamine and diglucosylpropylenediamine was also accomplished. In the reduction of the glucosylisopropylamine the *N*-alkyl-*d*-glucamine was contaminated with a reduced material believed to be sorbitol.

These amines were basic enough to be titrated potentiometrically. These compounds had the property of lowering the surface tension of water, and several were very good wetting agents.

Since the Amadori rearrangement was not easily effected with the alkylamines, a further attempt to obtain a rearranged compound was made by heating an excess of *n*-butylamine with glucose for several hours. Although quantitative tests showed that some enolization had occurred, no isoglucosamine compound could be recovered from the solution. Attempted rearrangement with an amide derivative of glucose showed that these compounds are even more stable to rearrangement than the alkylamine compounds.

The alkylamine derivatives hydrolyze about 40 per cent in a 2 per cent aqueous solution. The reaction was followed by change in rotation, potentiometric titration and extraction of the free amine at equilibrium. 1-Aminoglucose was more stable in water solutions but equally hydrolyzed by dilute acid. The aryl-*N*-glucosides showed a decided change in rotation, but after 90 hours only 10 per cent of the free amine was extracted. The abnormal behavior of the rotation of the *p*-toluidide of glucose in air and in a closed polarimeter tube indicated that changes other

than hydrolysis were occurring. The N-acyl-1-aminoglucose did not hydrolyze in water and was quite resistant to hydrolysis by dilute acids.

An attempt to prepare the amide derivatives of glucose by direct condensation was made, both with glucose and acetobromoglucose. Although there was evidence of reaction, no crystalline condensation product was obtained.

Weygand (3) postulated the Amadori rearrangement as part of the mechanism for osazone formation. Although the phenylhydrazine derivative of 2-methylglucose underwent a rearrangement with the loss of the methoxyl group and formation of glucose phenylosazone, as reported in the literature, the methoxyl group prevented an Amadori rearrangement of the *p*-toluidide of 2-methylglucose.

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CONSTRUCTION AND VALIDATION OF A SCALE FOR THE MEASUREMENT OF ATTITUDE TOWARD FARMING¹

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The development of desirable attitudes toward topics of social importance is recognized as one of the principal functions of education. Considered from the standpoints of legislation for agriculture, contributions to agricultural science, and efficiency in personal living by farm people, development of favorable attitudes toward farming is of social importance. Commitment to educational objectives necessitates and presupposes techniques for evaluating the attainment of the postulated objectives. This investigation was, therefore, undertaken in recognition of the need for an objective technique for evaluating farming attitude and for information concerning the farming attitude variable necessary in interpreting the results of evaluation.

This investigation was limited to the construction of a scale for measuring farming attitude, the relationship between farming attitude and age, and the possibility of breaking down farming attitude into two components, *viz.*, attitude toward farming as a vocation and attitude toward farming as a way of life.

A series of items of uncertain origin representing opinions toward farming was administered to students in the Divisions of Agriculture and Engineering at the Iowa State College. These items were the first draft of an experimental scale from which the final scale was developed. Evaluation of the experimental scale consisted of determining the suitability of the items from the standpoints of clarity, brevity, conciseness, inclusion of individual philosophy concerned with values or goals of living, and capacity to differentiate between groups of known different farming attitudes. The differentiating power of the items was determined in an objective fashion, *viz.*, noting the differences in the responses to each item by agricultural and engineering students. This item analysis suggested discarding certain items and revising others which were later included in a second draft of the experimental scale.

The simple method of scoring was utilized; *i. e.*, responses strongly favorable, favorable, neutral, unfavorable, and strongly unfavorable were given values of 5, 4, 3, 2, and 1, respectively.

Evaluation of the second experimental scale consisted of investigating validity, reliability, and sensitivity. Data for this purpose consisted of the performances on the scale by 1,918 boys enrolled in Iowa high schools serving rural youth. Reliability was determined by the split-half method, the coefficient of correlation between responses on odd and even items being .924 when stepped up by the Spearman-Brown formula. By means

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of the Z-function it was determined that the probability of obtaining an odd-even item correlation coefficient yielding a reliability coefficient less than .900 is practically negligible.

Validity was determined by showing that the scale differentiated between groups of boys of known different farming attitudes. Pupils intending to farm made significantly higher scores than those who did not intend to farm. Significant differences in the scores of vocational agriculture and other high school pupils and between 4-H and non-4-H boys were also obtained. The failure to find significant differences in the scores of vocational agriculture pupils and 4-H pupils indicated that the scale did not differentiate between groups having similar farming attitudes, and thus offered further evidence of the validity of the scale for Iowa high school boys.

A final scale was developed from the preliminary form administered to the Iowa high school boys. The changes in the final scale consisted of rearranging the items, deleting one item, and modifying the supplementary information called for on the test form. Satisfactoriness of the final scale was determined by its internal consistency, sensitivity, validity, and reliability.

Favorableness to farming as indicated by the entire scale was compared with favorableness to farming as revealed by each item and indicated that each item measured the same characteristic as the battery. Evidence of sensitivity was available from the lack of missing percentile values developed from the frequency distributions of scores made by Iowa high school boys.

The validity of the scale was determined by showing that it would differentiate between groups with known different farming attitude. For this purpose, scores were available for 2,198 boys enrolled in schools serving rural youth in Iowa, 380 Negro boys enrolled in Maryland high schools, 102 Virginia N. F. A. delegates, 56 Virginia State College agricultural students, 28 Princess Anne College students, and 206 freshman students enrolled in the Division of Agriculture of the Iowa State College. Comparisons of mean scores of these groups and of the percentiles of the mean scores indicated wide differences in farming attitude from group to group, thus providing evidence of the differentiating power of the scale. Analysis of the variance of scores in the Maryland group indicated that the variations of scores among schools was significantly greater than variations within schools. The Bartlett test of homogeneity failed to offer evidence that the significant F-tests were not the results of variations in mean scores from group to group. Differences in mean scores and the wide range of percentiles of the means from department to department at the Iowa State College offered further evidence of the differentiating power of the final scale.

Reliability of the scale was evaluated in each of the six groups. The reliability coefficients ranged from .883 to .942, evidencing a satisfactory degree of reliability.

The relationship of age and farming attitude was determined by the

technique of regression analysis. Linear and quadratic equations were fitted to the age-attitude data from each group. The total sum of squares was divided into two portions, one ascribable to regression and the other unexplained by regression. In all cases except one, the variation explained by linear regression was not significantly greater than that not associated with linear regression. In contrast, in all groups except Iowa vocational agriculture pupils, quadratic regression was statistically significant. By setting the first derivatives of each of the quadratic equations equal to zero and solving for X , the age was obtained at which boys between the ages of 13 and 19 were least favorable to farming. In all cases farming attitude was a minimum at approximately 16 years of age.

Investigation of the aspects of farming attitude consisted of scoring the items referring to farming as a vocation and the items referring to farming as a way of life as separate tests. The resulting two scales were evaluated for validity, reliability, and sensitivity as well as for their inter-relationship. Percentile norms, developed from the cumulative frequency distributions, indicated clearly that both scales are highly sensitive.

Reliability of the vocational and life scales was determined by the split-half method. The reliabilities differed from group to group, the ranges being .759 to .945, and .828 to .914 for the vocational and life scales, respectively. By means of analysis of variance it was shown that vocational and life scales each differentiate between groups of known different attitudes toward farming. Variation in the Maryland high schools was significantly greater among schools than within schools. Application of the Bartlett test of homogeneity failed to indicate that the significance of among-school variance over between-school variance resulted from differences in variability from school to school. Comparisons of the mean scores and of the percentiles of the mean scores of the six groups provided further evidence of the capacity of the scales to differentiate among groups.

The correlation coefficients between vocational and life scores in each group indicated that boys favorable toward farming as a vocation were also favorable toward farming as a way of life. Variation in the sizes of the correlation coefficients suggested that although for intra-group comparisons scores on one scale may be substituted for scores on the other, substitutions for inter-group comparisons are not necessarily defensible. In fact, the evidence available suggested that attitude toward farming as a vocation and attitude toward farming as a way of life are not identical behaviors from group to group.

Judging from the growing emphasis being placed upon attitude evaluation, the results of this study should have implications for classroom teachers, school administrators, and guidance officers. The scale, as a whole, or divided into vocational and life scales was found to be valid, reliable, and sensitive. It will be found useful whenever investigations of differences among individuals or groups are necessary or desirable.

EVALUATION OF MICROBIAL ACTIVITY IN SOIL PROFILES BY CARBON DIOXIDE EVOLUTION AND THERMAL PROCEDURES¹

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PART I

The purpose of the investigations was to establish some means of comparing the potential activities of soil populations and to ascertain whether the differences between the populations of the separate horizons of a profile are primarily numerical or essentially qualitative as well as quantitative.

Detailed studies were made of four soil profiles: Marshall and Clarion (Prairie), Marion (Planosol), and Fayette (Gray-brown Podzolic). Chemical analyses included total nitrogen, total carbon, and pH. Microbiological studies included ascertainment of CO₂ evolution from soil alone and soil plus cornstalks, and mineralization of soil nitrogen under optimum conditions. Water and alcohol extracts of soil were investigated for the possible presence of substances inhibitory to soil microorganisms.

A CO₂-rate procedure was developed for the comparative evaluation of soil populations, which takes into account not only the cumulative CO₂ evolution but also the time elapsing before the peak rate is attained. The peak rate of CO₂ evolution from soil alone invariably occurred during the first day of incubation, indicating that the population of each horizon was well adapted to the organic matter present. Although the total amount of CO₂ produced from the soil organic matter decreased rapidly with increasing depth, the differences among the horizons were not great if measured on the basis of percentage of carbon oxidized. The amount of nitrogen mineralized did not decrease consistently with depth nor was it consistently related to the carbon/nitrogen ratios of the soil horizons.

When available energy in the form of cornstalks was added at the rate of 1 per cent to the soils, in general the peak rate of CO₂ evolution was attained more and more slowly with increasing depth, indicating a progressive reduction in size of population with depth. Concurrently the magnitude of the rate peak was in most cases diminished, signifying a reduction in the potentialities of the population. This initial peak in CO₂ evolution from soil plus cornstalks was primarily at the expense of the water-soluble constituents.

The production of CO₂ from subsurface soil plus cornstalks was not much increased by inoculation with a surface soil population either by direct addition of soil or addition of soil suspension. Similar inocula in sand exhibited great activity. The failure of surface soil populations

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to develop normal activity in the subsoil may have been due to the presence of bacteriostatic substances in the latter.

Aqueous extracts of air-dried and fresh surface soils in general had no inhibitory effect on the growth of soil bacteria according to plate counts of a soil suspension and to pure cultural studies. The growth of bacteria in pure culture was measured with an Evelyn colorimeter. However, the alcohol extract of soil generally depressed the growth of certain soil bacteria and fungi on plates. The alcohol extract of an A₁ horizon had no influence on the counts of bacteria from a suspension of the same horizon, but caused a significant depression in the counts of soil suspensions of all horizons below it that were tested.

The presence in soil of bacteriostatic substances may keep the population of soils stabilized by preventing the ready development of introduced organisms. Apparently these inhibitory substances are produced in situ, because if they are produced in the A horizon and leached down to the B and C horizons, the bacteriostatic action should not be evident on inoculation with surface soil.

PART II

Heat, CO₂ and water are the main endproducts of microbial metabolic activity. The measurement of heat production or temperature rise is theoretically a more satisfactory method for the ascertainment of microbial activity than CO₂ production, since CO₂ production gives an incomplete impression of the decomposition processes. That is, compounds are not oxidized directly to CO₂ and water, but rather the oxidation is stepwise and with a mixed population not necessarily carried through to completion by one organism alone. These studies had the objectives of devising a thermal procedure to determine accurately yet simply the decomposition of organic materials in soil and of utilizing this procedure to ascertain microbial activity of soil populations.

The temperature changes of soils brought to optimum moisture content by addition of sucrose-nitrogen solutions were measured either galvanometrically or potentiometrically using copper-constantan thermocouples. In general 350 g. soil with 2.36 g. sucrose was placed in each of three pint jar style Dewar flasks. The experiments were run in a constant temperature room held within 0.1°C. of the desired temperature.

Evolution of heat due to heat of wetting phenomenon by soil colloids and cooling due to evaporation of water from the soil surface caused difficulties in selecting the proper base temperature from which to calculate temperature changes. The heat of wetting effect was nullified by employing fresh moist soil samples, or air-dry samples which previously had been placed in a saturated atmosphere. The net effect of water evaporation from the soil surface was reduced by using a large amount of soil in comparison with the volume of the Dewar flask.

The variation of the time-temperature curves amongst identically treated soils was somewhat greater than was to be desired, and only a part was accounted for by the small differences in the cooling curves

of the Dewar flasks. These variations between replicates are believed to be due to differences in the packing of the soil and distribution of moisture throughout the soil mass.

In a number of surface soils examined the maximum temperature change ranged from 2.5 to almost 7°C. and the time of maximum temperature ranged from 35 to 90 hours after moistening the soil.

The shape of the curves was interpreted in terms of microbial activity of the soil population. Soils that had been air-dried exhibited a lag period prior to the onset of temperature rise, which was probably due to the inactive state of the micro-organisms present in the soil. A high maximum temperature early attained indicated a large population capable of utilizing the energy material. On the other hand, a low maximum temperature without any pronounced peak temperature might indicate populations not so well able to utilize the energy material, or alternately this type of curve may be due to the sequence of organisms in the oxidation of the sucrose.

THE EFFECT OF VARIOUS ORGANIC RADICALS ON THE ATOMIC REFRACTIVITIES OF THE HALOGENS¹

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Small variations in the atomic refractivities of any given atom in a series of its compounds have long been observed, but few attempts have been made to correlate these variations with other properties of the compounds. Since most of the refraction of compounds arises from the interaction of light with the valence electrons, it seems that there should be an intimate relation between atomic refractions and the chemical bond.

This investigation was undertaken in an attempt to discover what effect, if any, organic radicals exert on the refractions of the atoms with which they are combined. A series of organic iodides, bromides, chlorides, and their parent hydrocarbons was chosen for study since it was felt that any order of radicals obtained on the basis of the effect on one halogen should be capable of being checked with the other halogens. It was also sought to determine if the order obtained was independent of the wave-length of light used. To do this, the molar refractions, as defined by the Lorentz-Lorenz formula

$$R_{\lambda} = \frac{n_{\lambda}^2 - 1}{n_{\lambda}^2 + 2} \frac{M}{d},$$

were calculated for each of seven visible spectral lines for each substance. The seven spectral lines used were: H_a, 6563; Na D, 5893; Hg yel., 5770; Hg grn., 5461; H_β, 4861; Hg blue, 4358; and Hg viol., 4047.

Dispersion equations of a modified form of the Sellmeier equation,

$$n^2 = a + \frac{b}{c - \nu^2},$$

in which the three constants, a, b, and c, were evaluated by choosing values of n corresponding to three frequencies of light, were fitted for each compound, and the molar refractions at infinite wave-length were calculated.

Refractive index measurements were made with a small, hollow prism on a spectrometer, the scale of which could be read to the nearest 20 seconds. The density measurements were made with capillary pycnometers of about 10 milliliter capacities.

The atomic refractions of the halogens were calculated by subtracting the measured molar refractions of the parent hydrocarbons, less

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the atomic refraction of hydrogen, from the measured molar refractions of the halogen compounds.

The order of radicals with the liquid compounds, benzyl, p-tolyl, phenyl, and n-heptyl, with the atomic refractions of the halogens being highest with the first named, was established and maintained for each spectral line including that of infinite wave-length. The same order was also obtained when the atomic dispersions for the halogens were compared.

The molar refractions of the ortho- and meta-halonitrobenzenes were measured in dioxane solutions at 0.2 and 0.4 mol fraction concentrations. It was found that the atomic refractions of the halogens are always higher for the meta compounds at all wave-lengths of light including that of infinite wave-length. The molar refractions are always appreciably higher in the more dilute solutions.

Table 1 gives the boiling points, the densities at 20.0° C. calculated

TABLE 1
PHYSICAL CONSTANTS OF THE LIQUIDS

Compound	b. p. °/mm.	d _{vac.} ²⁰	n _D ²⁰	R _D	R _∞
Benzene	79.8/743	0.87774	1.50114	26.220	25.129
Chlorobenzene	131.0-.1/739	1.10648	1.52440	31.144	30.002
Bromobenzene	156.0/740	1.49543	1.55948	33.925	32.561
Iodobenzene	188.0/737	1.82903	1.62086	39.226	37.460
Toluene	109.9/742	0.86591	1.49711	31.141	29.916
p-Chlorotoluene	160.7/736	1.06930	1.52047	36.016	34.648
p-Bromotoluene	184.0/743	1.39296*	1.54748*	38.968	37.452
p-Iodotoluene	210.7/735	1.67267†	1.59509†	44.300	42.310
Benzyl chloride	177.7/741	1.09921	1.53879	36.064	34.729
Benzyl bromide	197.0/734	1.43845	1.57671	39.388	37.689
n-Heptane	97.5/734	0.68380	1.38807	34.582	33.803
n-Heptyl bromide	37.2/3mm.	1.13910	1.45027	42.277	41.211
n-Heptyl iodide	203.4/739	1.38001	1.49085	47.441	46.003
Nitrobenzene	209.1/740	1.20306	1.55203	32.696	31.160

* at 30°. † at 40°.

to a vacuum, the refractive indices for the Na D line and the light of infinite wave-length for each of the liquids studied.

Table 2 gives the melting points, and the molar refractions for the Na

TABLE 2
PHYSICAL CONSTANTS OF THE SOLIDS

Compound	m.p.	0.2 mol frac.		0.4 mol frac.	
		R _D	R _∞	R _D	R _∞
m-Chloronitrobenzene	45.0	38.208	35.970	38.089	36.225
m-Bromonitrobenzene	55.5	41.038	38.950	40.957	38.937
m-Iodonitrobenzene	37.7-38.0	46.281	44.065	46.090	43.608
o-Chloronitrobenzene	31.7-.9	37.899	35.863	37.674	36.140
o-Bromonitrobenzene	38.5-39.0	40.762	38.499	40.589	38.280
o-Iodonitrobenzene	49.0-.2	45.918	42.863	43.682	42.439
Nitrobenzene	b.p. 209.1/740	33.109	31.621	32.894	31.168

D line and light of infinite wave-length at 0.2 and 0.4 mol fraction concentrations for each of the solids studied.

Tables 3 and 5 give the atomic refractions of the halogens and their differences in the liquids and solids, respectively.

TABLE 3
ATOMIC REFRACTIVITIES OF THE HALOGENS AND THEIR DIFFERENCES IN
THE LIQUIDS FOR THE Na D LINE

Radical	I	Br	Cl	I-Br	I-Cl	Br-Cl
Benzyl		9.348	6.024			3.324
p-Tolyl	14.260	8.928	5.976	5.332	8.284	2.952
Phenyl	14.106	8.805	6.024	5.301	8.082	2.781
n-Heptyl	13.959	8.795		5.164		

Table 4 gives the atomic dispersions of the halogens and their differences for the region between the H_a line, 6563 Å, and the Hg blue line, 4358 Å, for each of the liquids.

TABLE 4
DISPERSION OF ATOMIC REFRACTIONS BETWEEN Hg BLUE AND H_a LINES OF THE HALOGEN
ATOMS AND THEIR DIFFERENCES

Radical	I	Br	Cl	I-Br	I-Cl	Br-Cl
Benzyl		0.555	0.215			0.340
p-Tolyl	0.899	0.395	0.226	0.504	0.673	0.169
Phenyl	0.878	0.387	0.215	0.491	0.663	0.172
n-Heptyl	0.780	0.352		0.428		

Although it seems significant that the same order of radicals is maintained when effects on the atomic refractions of each halogen and their dispersions are considered, no linear relationship is observed between this and "electron-sharing ability," Kharasch's electronegativity series, molecular weight, or chemical reactivity. Atomic refraction is undoubtedly due to a complex combination of various forces acting in different directions.

TABLE 5
ATOMIC REFRACTIVITIES OF THE HALOGENS AND THEIR DIFFERENCES IN
THE SOLIDS WITH THE Na D LINE

Radical	I	Br	Cl	I-Br	I-Cl	Br-Cl
<i>0.2 mol fraction</i>						
m-Nitrophenyl	14.272	9.029	6.199	5.243	8.073	2.830
o-Nitrophenyl	13.909	8.753	5.890	5.156	8.019	2.863
<i>0.4 mol fraction</i>						
m-Nitrophenyl	14.296	9.163	6.295	5.133	8.001	2.868
o-Nitrophenyl	13.888	8.795	5.880	5.093	8.008	2.915

OXYGEN CONSUMPTION AT VARIOUS TEMPERATURES BY NYMPHS AND ADULTS OF THE GRASSHOPPER, *MELANOPLUS DIFFERENTIALIS* (THOMAS)¹

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A review of the literature of respiratory metabolism of insects is presented and accompanied by a tabulated summary of measurements of the respiratory metabolism of insects for the period 1914 through 1941.

The writer used the grasshopper, *Melanoplus differentialis*, as the experimental insect. Eggs of *M. differentialis* collected in the field were identified in the laboratory according to the method of Tuck (1939). After the diapause was broken, the eggs were placed in damp sand and incubated in a constant temperature cabinet operating at 30° C. The nymphs and adults were reared in Riley cages placed in a heated, sunlit greenhouse; they fed on potted plants of barley, wheat, corn, and alfalfa placed in the cages.

The instars were separated according to the key for *Melanoplus bivittatus* devised by Shotwell (1941), and each instar was kept separate from the others.

The instrument used to measure the oxygen uptake was the Warburg manometer, a constant volume type of respirometer. Three sizes of Warburg flasks, 5 cc., 15 cc. and 150 cc., with central well were used. First and second instar nymphs were placed in 5 cc. flasks. The 15 cc. flasks were used for third and fourth instar nymphs; and 150 cc. flasks were used for fifth instar nymphs and adults. The Warburg manometers were used according to directions given in Dixon (1934).

The Warburg manometers were suspended on the side of a water bath so that the flasks were immersed in the water during the intervals used to measure the oxygen uptake. Temperature control of the water bath was accomplished through the use of a quick-set bi-metallic thermostat. Three blade type heating elements provided the heating unit. When temperatures below that of room temperature were desired, a cooling unit consisting of a pump to circulate water from a cold coil, surrounded by cracked ice, outside the bath to a coil in the bath through the pump and back to the cold coil was used.

The volume of the insects introduced into the flask, needed for calculation of the manometric constant, was determined by the use of a volumenometer. A figure showing details of the volumenometer is provided and directions for its use are given.

According to Parker and Shotwell (1932) and Shotwell (1941) the threshold temperature for spontaneous movement in *Melanoplus differentialis* occurs at 68° F. In order to study the relationship of rate of

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metabolism to the threshold temperature for spontaneous movement, the oxygen uptake was measured for each nymphal instar and for young adults of *M. differentialis* at the controlled temperature of 40°, 50°, 60°, 65°, 70°, and 80° F.

The number of grasshoppers which could be accommodated without crowding in a manometric flask suitable to their size was determined to be ten first instar or five second instar nymphs in a 5 cc. flask, five third instar or three fourth instar nymphs in a 15 cc. flask and five fifth instar or two adults in a 150 cc. flask. The use of several individuals in a respirometer flask resulted in average readings, but enabled the author to use a short interval for the measurement of oxygen uptake. Also, it was felt that fluctuations between respirometers would not be as great as if single individuals were used.

Groups of grasshoppers to be used in the measurement of oxygen uptake were selected at random from a stock cage. They were weighed to 1 mg. on a balance accurate to 0.1 mg.

Care was taken to avoid injuring the grasshoppers while handling them in selection, weighing, and placing them in the respirometers.

After the grasshoppers were placed in a respirometer properly arranged to measure oxygen uptake, the respirometer was placed on the water bath. The stopcock was left open for 30 minutes to allow equalization of pressure within the respirometer due to change in temperature from that of room temperature to that of the water bath. This interval also gave the grasshoppers an opportunity to become accustomed to their surroundings, recover from the excitation of being handled, and quiet down.

At the end of the 30 minutes the liquid in the manometer was adjusted to the 150 mm. mark, and the stopcock closed. During the following 15 minutes any carbon dioxide which might have been present in the air or produced by the grasshoppers during the preliminary 30-minute conditioning period was taken up. At the end of this 15-minute interval the stopcock was left closed; the level of the liquid in the right-hand arm of the manometer was adjusted to the 150 mm. mark, and the reading of the liquid taken in the left-hand arm of the manometer. Observation of the respiratory metabolism was taken for the following 15-minute interval.

After correction by the manometric constant, the results were recorded as oxygen uptake in cmm./mg./15 min.

In addition to a study of the relationship of the rate of metabolism to the threshold temperature for spontaneous movement, the oxygen uptake of adult *Melanoplus differentialis* males and females was measured at 80° F. at weekly intervals from the beginning of adulthood until death. Also, measurements were made of the oxygen uptake at 80° F. of adult male and female *Periplaneta americana* (Linn.), *Brachystola magna* (Scudd.), and *Schistocerca lineata* Scudder, and of female *Scudderia furcata* Brunner.

The results are presented in tables. Graphs showing the oxygen up-

take of each instar at various temperatures and the oxygen uptake of the various instars at each temperature are given. A summary of results is presented in Table 1.

TABLE 1
MEANS OF OXYGEN UPTAKE IN CMM./MG./15 MIN. OF NYMPHS AND ADULTS OF *Melanoplus differentialis* AT VARIOUS TEMPERATURES

Instars	Temperatures in Degrees Fahr.					
	40	50	60	65	70	80
First.....	.026 ± .006	.067 ± .006	.095 ± .016	.153 ± .041	.219 ± .015	.376 ± .033
Second.....	.031 ± .006	.051 ± .007	.104 ± .012	.145 ± .010	.197 ± .018	.338 ± .050
Third.....	.024 ± .004	.033 ± .007	.092 ± .008	.105 ± .013	.160 ± .003	.264 ± .028
Fourth.....	.021 ± .007	.042 ± .012	.078 ± .013	.115 ± .009	.156 ± .025	.274 ± .025
Fifth.....	.023 ± .009	.046 ± .005	.069 ± .014	.104 ± .016	.124 ± .025	.189 ± .038
Young adult						
Males.....	.021 ± .005	.023 ± .003	.059 ± .009	.072 ± .006	.083 ± .033	.160 ± .030
Females.....	.025 ± .008	.028 ± .002	.072 ± .012	.101 ± .031	.130 ± .057	.199 ± .031

The following conclusions may be drawn.

1. The oxygen uptake of the five nymphal instars of *Melanoplus differentialis* increases at a uniform rate with increase in temperature between 40° and 80° F.

2. No significant difference is shown in the means of oxygen uptake of adult *Melanoplus differentialis* at 40° and 50°.

3. The rate of oxygen uptake indicates that a sudden increase or significantly higher rate of metabolism is not associated with the threshold temperature for spontaneous movement in *Melanoplus differentialis*; or that as the temperature approaches the threshold temperature for spontaneous movement the rate of metabolism does not increase more than would be expected from the change in temperature.

4. The rate of oxygen uptake at 80° F. during the growth of *Melanoplus differentialis* from first instar to adult is in agreement with Rubner's Surface Law.

5. Contrary to the expectations in accordance with Rubner's Surface Law the means of oxygen uptake of instars of *Melanoplus differentialis* at 40° F. do not differ significantly.

6. The oxygen uptake of fourth instar nymphs of *Melanoplus differentialis* at each of 65°, 70°, and 80° F. is higher than would be expected from the surface law relationship.

7. During the first two weeks of adult life, female *Melanoplus differentialis* have a higher oxygen uptake than males of the same age; after adult *Melanoplus differentialis* are three weeks old the oxygen uptake of males is higher than that of females.

8. The males of *Schistocerca lineata*, *Brachystola magna* and *Periplaneta americana* have a slightly higher rate of metabolism at 80° F. than do the females.

9. A significant decrease at the third week is shown in the means of

oxygen uptake of females *Melanoplus differentialis* measured at successive weekly intervals during adult life. It is suggested that this is perhaps due to the high rate of oxygen uptake at the beginning of adult life in the development of the ovaries and formation of the eggs followed by the gravid condition and beginning of egg-laying activities at about the third week of adult life.

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FACTORS ASSOCIATED WITH THE RETENTION OF CARBON DIOXIDE IN CARBONATED BEVERAGES¹

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Carbon dioxide dissolved in water forms the basis of most carbonated beverages. As held under gas pressure in bottles or containers, the amounts of carbon dioxide present in the liquid are in excess of the normal solubility at atmospheric pressure. Brought to atmospheric pressure on opening the container, the carbon dioxide is released, until equilibrium is reached at the solubility corresponding to the existing temperature. The carbon dioxide liberation is not instantaneous, but varies in rate apparently with the attendant circumstances. Hence, the retention, or conversely, the rate of release of this ingredient has been a subject of interest and unorganized speculation for many years.

A study was made to investigate the retention of carbon dioxide in liquids commonly employed in carbonated beverages, under the conditions generally associated with their preparation and use. The amounts of carbon dioxide present are measured as volumes, the same solubility factor as Bunsen's *alpha*, which indicates the volumes of gas, corrected to 0°C. and 760 mm. of mercury, per volume of water. Measurement of the gas content may be made by piercing the metallic closure of a bottle with a hollow needle attached to a pressure gauge, and interpreting this observed pressure reading into volumes of carbon dioxide by indexing against the temperature of the bottle contents on a standard chart. Such charts have been based on observed solubilities of carbon dioxide at atmospheric pressure within the temperature range of 32° to 100°F., computing solubilities at higher pressures as multiples of those at one atmosphere, assuming the applicability of the ideal gas laws in this instance.

A carefully calibrated stainless steel bomb was used to duplicate the conditions found in a closed container; it also served as a very excellent means for absorbing carbon dioxide under pressure in the liquids in question. The accepted solubilities of carbon dioxide in water at atmospheric pressure were checked within the range of 32° to 100°F., and found accurate. Other factors were believed to be acting at pressures above atmospheric, and a series of temperature-pressure relationships were investigated, checking the actual carbon dioxide content in each run by removing it from the liquid and measuring its volume.

From such observations it would be possible to predict accurately the amount of carbon dioxide present in a given closed system by measuring the developed gas pressure at a known temperature. In a closed container, the total gas is divided between that compressed in the top space above the liquid and that absorbed in the liquid. With rising temperature, carbon

¹ Original thesis submitted December 10, 1937. Doctoral thesis number 449.

dioxide appears to migrate into the top space, probably due to the solubility decrease proceeding more rapidly than the compressibility of the gas with temperature rise. This phenomenon was studied with carbon dioxide contents of 2 to 5 volumes and with ratios of top space to total container volume of 1 in 30 minimum to 1 in 5 maximum.

It was found that the size of the top gas space above the liquid in the container exerted a marked effect, lowering the resulting pressures with temperature rise as the relative size of the top space was increased. Air present in the top space as an impurity in the carbon dioxide exerted an additive pressure above the normal without appreciable increase in the total gas dissolved in the liquid because of the low relative solubility of air in water. Sucrose in solution decreased the solubility of carbon dioxide in the liquid in direct proportion to the amounts present and exerted a slight lowering effect on the temperature-pressure relationships. Small amounts of mineral salts and flavoring agents, not including sugar, showed no appreciable effect on the temperature-pressure relationships.

Consideration of these observed temperature-pressure relationships indicated the possibility of computing the values by mathematical formulae allowing compensation for the relative top spaces, from which corrections could be applied to the theoretical standards used in industry. The effect of the top space was found to be independent of the size of the container but correlated with the ratio of the top space to the total volume of the container. The air content could be expressed as an imposed pressure above that of the carbon dioxide. Sucrose in solution could be compensated by adjustment of the top space ratio, since it appeared that the sucrose was displacing a portion of the active absorbing water.

The amounts of carbon dioxide in varying combinations of liquid and top space being readily predictable, it was possible to use these readings as the basis for the study of the gas retention by the liquid on the release of pressure. The bomb used was fitted with a side arm valve, which would allow controlled release of the gas to a measuring system. Displacement flasks consisting of balanced liquid systems were employed, which allowed the evolved gas to displace an inert saline solution at a constant back-pressure. The displaced saline solution could then be measured readily and accurately. It was observed that the evolution of carbon dioxide could be shown as a straight line function of the logarithm of time when plotted graphically, and that the slope of this line would serve as an index to the rate of evolution for any given run.

Under the conditions of observation, a number of relationships appeared reasonable. As the amounts of absorbed carbon dioxide were increased, the evolution rates from the liquid increased regularly with evidence of a range of instability beyond which the evolution rates increased disproportionately. As the temperature of evolution from the liquid was increased, the rate of evolution also increased in relatively proportionate manner, due probably to increase in the gas tension in the liquid and increase in the rate of diffusion of gas through the liquid to points of bubble formation. Air in the gas was found to decrease the

stability of the absorbed carbon dioxide, promoting rates of evolution in excess of the proportionate amounts of air present. Sucrose in the solution lowered the solubility and possibly the rate of diffusion of the gas in the liquid, lowering the evolution rates, while other common flavoring ingredients showed no appreciable effect.

In considering the mechanism of gas release from the liquid, it was observed that nuclei for bubble formation were extremely important, since without bubble formation the rates of gas loss by the mechanism of diffusion through the surface were relatively negligible. The number and distribution of physical nuclei showed a proportionate trend to the rates of evolution found, so that in most cases at a given temperature the rate of evolution observed will be a combined function of the total carbon dioxide content and the active nuclei on the container walls or distributed through the liquid.

In practice, it will be possible to predict more accurately the amounts of carbon dioxide present in any given system or container through the corrected temperature-pressure relationships established, and, by control of the action of nuclei, it will be possible to obtain retention characteristics as desired for any given carbon dioxide content.

CHANGES PRODUCED IN GROWTH, REPRODUCTION, BLOOD, AND URINE OF RATS BY INGESTION AND ORAL ADMINISTRATION OF COBALT SALTS¹

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A study was made of the effects upon growth, reproduction, and lactation of rats fed an adequate basal ration to which cobalt chloride or cobalt nitrate had been added. Concentrations of 25 parts per million or less of cobalt added to the diet were found to be without appreciable effect on the growth rate, success of lactation, and reproduction of the first litter. When 50 parts per million of cobalt were added to the diet, growth of rats was decreased, primarily due to the decreased feed intake; the number of young per litter, the weight of each litter, and the average weight of each young were all decreased, and success in lactation was decreased to a small extent. With concentrations of 100 parts per million or more of cobalt added to the diet, reproduction ceased entirely. With 200 parts per million of cobalt in the diet, growth was decreased both because of decreased feed intake and because of some other toxic effect of the cobalt salt within the organism. The decreased growth rate caused by 200 parts per million of cobalt in the diet was not due to any permanent injury to the rat (as far as growth was concerned) since growth was resumed when the cobalt salt was omitted from the diet. In all cases, the decreased growth caused by adding cobalt salts to the diet was approximately proportional to the concentration of cobalt salt fed. When concentrations of 500 parts per million of cobalt were fed to young rats, they died within 3 to 5 weeks. Higher cobalt concentrations in the ration were lethal in a shorter length of time.

Cobalt analyses were made on tissues of rats fed cobalt salt and on tissues of rats which had not received added cobalt salt in their diet. In general, the tissues of rats not fed cobalt salts contained less than 1 part per million of cobalt. The tissues of rats fed cobalt salt showed marked increases in cobalt content in the liver and kidneys with lesser increases in the spleen, pancreas, heart, lungs, and testes. The least increases in cobalt content occurred in the intestine and the stomach. Only in the liver was the concentration of deposited cobalt proportional to the concentration of cobalt fed in the diet. Deposition of cobalt in the kidneys was less than in the liver but greater than in the other organs studied. When young rats were fed a diet containing 100 parts per million of added cobalt (as cobalt nitrate), the concentration of cobalt deposited in the liver reached its maximum value in 4 to 5 days after the feeding of such a ration was started.

When rats were fed 50 or 200 parts per million of added cobalt and

¹ Original thesis submitted May 26, 1943. Doctoral thesis number 716.

control groups were fed the same weight of the basal ration as consumed by the rats receiving cobalt, the amount of reducing sugar in the urine of the rats fed cobalt salt was almost twice that of the controls not fed added cobalt. Albumin was present in the urine of rats fed cobalt salt as well as in that of rats not fed cobalt salt. The concentration of non-protein nitrogen in the blood was apparently not altered by feeding cobalt salt under these circumstances.

Ten parts per million of cobalt (as cobalt nitrate) in the diet seemed to be the lowest concentration that would stimulate hemoglobin formation, and this stimulation was not permanent, the hemoglobin concentration decreasing after about 14 weeks to the level of the controls not fed added cobalt salt.

When cobalt nitrate or cobalt chloride solutions were injected by stomach tube into the stomachs of anesthetized rats, the minimum lethal dose was found to be approximately 20 milligrams of cobalt per 100 grams of body weight. There was little difference in the toxicity of the two salts.

When less than the lethal amount of cobalt salt was injected by stomach tube, the blood sugar concentration increased markedly in 30 to 60 minutes after the injection and increased still more from 60 to 120 minutes after the injection. The effect was more pronounced when more concentrated solutions were injected, even though the same amount of cobalt was given. The hyperglycemia was not due to the slight acidity caused by hydrolysis of the cobalt chloride. When 200 milligrams of glycine were injected by stomach tube along with 20 milligrams of cobalt, no such increase in blood sugar concentration occurred, the glycine apparently detoxifying the cobalt salt. When glucose solutions were injected into the stomach with cobalt salt in a similar manner, the normal decrease in blood sugar concentration in 60 to 120 minutes after injection (expected of glucose injections alone) did not occur, the blood sugar concentration remaining at a constant, elevated value during the 60- to 120-minute period after injection. Apparently the normal blood sugar decrease expected from the glucose injection was just offset by the hyperglycemia caused by the toxic action of the cobalt salt.

When glycine and cobalt chloride were fed to rats in the growing ration, glycine completely failed to prevent the cobalt salt from decreasing growth and stopping reproduction. In addition, the added glycine failed to prevent the added cobalt salt from increasing the hemoglobin concentration and the erythrocyte count in the blood.

FORMS OF INORGANIC PHOSPHORUS IN THE LOWER HORIZONS OF SOME IOWA SOILS AS INDICATED BY PLANT AVAILABILITY AND CHEMICAL METHODS¹

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The productivity of subsoils is well known to be less than that of their respective surface soils, and moreover, the ability of various crops to thrive on subsoils differs noticeably. Of the several theories which have been advanced to explain the relative unproductivity of subsoils, the deficiency of phosphorus in a form available to plants has been given considerable attention.

The amounts of total and dilute acid-soluble phosphorus have been found to increase with depth in the soil profile below the surface horizon, excepting for a few cases. Crop yields, however, are not in step with these increases, and it appears that the subsoil phosphorus may be relatively unavailable. The form of phosphorus present in seven Iowa subsoils has been investigated, directly by plant availability determinations in the greenhouse, and indirectly by solubility studies and comparisons with known phosphorus-bearing minerals in the laboratory.

Soil samples from several depths of the soil profile were obtained, treated, potted in 2-gallon earthenware pots, and planted in triplicate to various crops. The average dry weight for each treatment was used as an indication of phosphorus availability, since potassium and nitrogen were added to all soil samples. The soils used were Weller, Fayette, Tama, Marshall, Grundy, Clarion, and Shelby.

The laboratory investigations were made on the C horizon of the same soils which were used in the greenhouse, and on Tennessee Brown Rock Phosphate, apatite, vivianite, dufrenite, wavellite, variscite, Volclay bentonite, and kaolinite receiving various treatments. Phosphorus was extracted during 24 hours with solutions having the pH adjusted over a wide range with dilute HCl and NaOH. Certain subsoils were also extracted during 30 minutes and at varying soil to .002 N H₂SO₄ solution ratios. On the basis of comparisons of the phosphorus solubility characteristics of subsoils and minerals and of the greenhouse evidence, interpretations were made as to the forms of phosphorus probably present in the subsoils.

The results may be summarized as follows: (1) Grass crops grown on the Grundy C horizon gave greater response to phosphate fertilization than calciphilic legumes even though a high content of dilute acid-soluble phosphorus was present. Corn and alfalfa increased in yield as a result of phosphate fertilization of calcareous Clarion subsoil which contained a moderate amount of dilute acid-soluble phosphorus; however, the phos-

¹ Original thesis submitted July 10, 1942. Doctoral thesis number 673A.

phorus requirement of alfalfa was satisfied with a smaller application than was that of corn. The relatively high pH of the subsoils makes the presence of tricalcium phosphate possible. Improvement of the aeration and drainage conditions in Grundy subsoil did not affect crop yields. The curves representing the phosphorus extracted from Grundy and Clarion subsoils over a wide pH range are very similar to those of calcium phosphates in that they indicate high solubility of phosphorus in acid solutions and low solubility at high pH values. An apatite or apatite-like form of phosphorus probably exists in these two subsoils as indicated from the observations stated above.

(2) Tama and Marshall C horizons are slightly acid and contain high amounts of dilute acid-soluble phosphorus which is not available to grass crops but is available to calciphilic plants, especially alfalfa. To satisfy the phosphorus need of sudan grass, a very large phosphate application was necessary; alfalfa was able to use the native supply. Corn and sudan grass responded to phosphorus fertilization on Tama, whereas alfalfa, sweet clover, and red clover did not. Addition of the surface soil of Marshall silt loam to the subsoil resulted in increased plant growth probably owing to the release of phosphorus. Addition of undecomposed organic matter was of no advantage to the growth of the first two crops but gave an increase with the third crop. The dilute acid-soluble phosphorus was reduced upon adding organic matter. Acidifying the soil before growing crops increased phosphorus availability as indicated by the reduced response to phosphorus fertilization and much greater yields of sudan grass and corn. Liming had no effect on yields. Crop yields on Marshall C horizons were substantially increased by the addition of silica gel but not by improvements in aeration and drainage. The phosphorus solubility data for Tama and Marshall subsoils were not markedly similar to those for any one mineral; however, similar results could be expected from the presence of a mixture of apatite and vivianite since both these minerals exhibit high phosphorus solubility below pH 5.5 and vivianite yields large amounts of soluble phosphorus at pH above 7.0. A rapid decrease in the phosphorus concentration of extracts was obtained as the soil to acid extractant ratio widened, thereby indicating a rapid exhaustion of an acid-soluble form of phosphorus. These results indicated the presence of a mixture of tricalcium phosphate and vivianite in the Tama and Marshall C horizons. However, the possibility of having tricalcium phosphate supplemented with aluminum phosphates cannot be fully discarded without further investigations.

(3) The phosphorus in the C horizon of Weller and Fayette subsoils, which contained a large amount of dilute acid-soluble phosphorus, was equally available to grasses and alfalfa. Liming caused a slight decrease in the availability of the phosphorus to the first grass crop but not to alfalfa; silica gel had no appreciable effect on Fayette subsoil phosphorus available to plants. Liming increased the amount of dilute acid-soluble phosphorus in both the Weller and Fayette subsoils, whereas silica gel had no appreciable influence on the dilute acid-soluble phosphorus in Fayette

subsoil. Undecomposed organic matter had no substantial influence on plant growth nor on the acid-soluble phosphorus in Fayette subsoil. Large amounts of phosphorus in these two subsoils were soluble at high and low pH values which is in agreement with the solubility of vivianite phosphorus. The soil reactions of Weller and Fayette C horizons are pH 5.0 and 5.2 which suggest the absence of tricalcium phosphate as the principal form. These data indicate the presence of vivianite as the form of phosphorus in Weller and Fayette C horizons.

(4) The Shelby C horizon has a pH of 5.48 and a low amount of dilute acid-soluble phosphorus which exclude the possibility of tricalcium phosphates being the principal form of phosphorus. Sudan grass and alfalfa respond to phosphorus fertilization and make little growth on this subsoil. The phosphorus concentration of extracts from a 30-minute extraction of subsoil with increasing soil to .002 N H_2SO_4 solution was very low and decreased very slowly, which indicated the presence of an insoluble basic ferric phosphate. The amounts of phosphorus extracted from Shelby subsoil and colloid during 24 hours in acid solutions were very low and did not substantially increase below pH 7.0. The solubility data of Shelby subsoil phosphorus are very similar to dufrenite phosphorus. These facts warrant the description of the phosphorus present in the C horizon of this soil as a dufrenite-like form.

AZO LEAD DYES¹

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As a preliminary to the preparation of the azo lead dyes described in this work, it was necessary to make some halogen-metal interconversion studies.

n-Butyllithium was used in all of the interconversions. When *n*-butyllithium was prepared at 0° rather than at room temperature, the time of preparation was reduced considerably, and the yield was increased.

When one equivalent of *o*-bromoaniline was allowed to react with three equivalents of *n*-butyllithium for 45 minutes at room temperature, the yield of interconversion product was 40 per cent. *m*-Bromoaniline was almost unaffected under the same conditions. Likewise, *p*-iodoaniline and 4-iodoresorcinol did not undergo interconversion to any appreciable extent.

Two equivalents of *n*-butyllithium and *p*-bromo-*N*-methylaniline gave, subsequent to carbonation, a 27 per cent yield of *p*-methylaminobenzoic acid. When *N*-(*p*-iodophenyl)-phthalimide was allowed to react with *n*-butyllithium at -50° for 70 minutes and the mixture was carbonated, the yield of *N*-(*p*-carboxyphenyl)-phthalimide was 37 per cent.

The best procedure for preparing an organolead compound from an organolithium compound and a triaryl- or trialkyllead halide was to add the organolithium compound to a suspension of the aryllead halide in ether and then to hydrolyze the mixture after approximately 5 minutes. The reaction between an organolead halide and an organolithium compound is almost instantaneous. A short reaction time greatly reduces the formation of by-products. Under these conditions *p*-aminophenyllithium and triphenyllead chloride yielded triphenyl-*p*-aminophenyllead (m.p. 172°); *o*-aminophenyllithium and triphenyllead chloride gave triphenyl-*o*-aminophenyllead (m.p. 164-5°); and *p*-methylaminophenyllithium and triphenyllead chloride reacted to produce triphenyl-*p*-methylaminophenyllead (m.p. 97-98°). *o*-Dimethylaminophenyllithium, prepared directly from *o*-bromo-*N,N*-dimethylaniline and lithium, and triphenyllead chloride, resulted in triphenyl-*o*-dimethylaminophenyllead (m.p. 101°).

o-Hydroxyphenyllithium, obtained by the interconversion of *o*-bromophenol with *n*-butyllithium, reacted with triphenyllead chloride to give triphenyl-*o*-hydroxyphenyllead (m.p. 217-218° d.). *p*-Hydroxyphenyllithium and triphenyllead chloride failed to give the desired organolead compound. Warming a suspension of diazotized triphenyl-*p*-aminophenyllead in water yielded a small amount of material melting at

¹ Original thesis submitted March 15, 1943. Doctoral thesis number 711.

230°. The lead content of this material was 1 per cent lower than that calculated for triphenyl-*p*-hydroxyphenyllead.

p-Dimethylaminophenyllithium prepared from *p*-bromo-*N,N*-dimethylaniline and lithium, and triethyllead chloride yielded triethyl-*p*-dimethylaminophenyllead. The later compound had the following physical constants: b.p./1 mm., 130°; n_D^{25} , 1.5442; and D_4^{25} , 1.4982.

Under varying conditions triethyllead chloride was allowed to react with the interconversion products of *n*-butyllithium and *o*-bromophenol, *p*-bromoaniline, and *p*-bromo-*N*-methylaniline. Likewise, the interconversion products of *n*-butyllithium and *o*-bromophenol and *o*-bromoaniline were tried with trimethyllead chloride. Attempts to isolate the desired pure products from any of these reactions failed. The difficulty probably resides in the separation of the reaction products from the unreacted materials. At atmospheric pressure the alkyllead compounds are unstable at their boiling points. At reduced pressure the boiling points of the desired organolead compounds and the unreacted amine or phenol are too close together to permit separation.

N-(*p*-lithiophenyl)-phthalimide and triethyllead chloride gave a light-yellow, glasslike material, the lead analysis of which corresponded to the calculated value for triethyl-*p*-(*N*-phthalimido)-phenyllead. Alkaline hydrolysis of the latter compound yielded phthalic acid and a gummy residue which could not be purified.

When triphenyl-*p*-aminophenyllead, triphenyl-*o*-aminophenyllead, triphenyl-*o*-dimethylaminophenyllead, triphenyl-*o*-hydroxyphenyllead, and triethyl-*p*-dimethylaminophenyllead, respectively, were dissolved in chloroform and treated with dry hydrogen chloride, the substituted aryl group was cleaved predominantly. Triphenyllead chloride and the cleaved group were both recovered and identified in each reaction.

When the reaction between triphenyllead chloride and the *C*-lithioanilines was allowed to continue for several hours, considerable amounts of tetraphenyllead were always formed. The same was true of the reaction between *p*-bromo-*N*-lithioaniline and triphenyllead chloride. The tetraphenyllead could possibly result from the disproportionation of an unstable compound containing a lead-nitrogen linkage. A long-time reaction between triphenyllead chloride and *o*-lithiolithium phenoxide resulted in considerable quantities of triphenyllead carbonate. This may be formed by the hydrolysis of triphenyl-*o*-lithiophenoxylead to triphenyllead hydroxide. Triphenyllead hydroxide in contact with the carbon dioxide of the air would give triphenyllead carbonate.

Two methods were used for the preparation of organolead compounds containing an azo linkage joined to the lead atom through carbon atoms. First, aminoaryllead compounds were diazotized and coupled with various hydroxyl- and aminoaryl compounds. Second, primary amines were diazotized and coupled with organolead compounds amenable to diazo coupling. The first method is not desirable from a preparative point of view, while the latter method gave good yields.

When triphenyl-*p*-aminophenyllead was dissolved in glacial acetic

acid, cooled to 15°, then diazotized by the addition of sodium nitrite, and the resulting mixture was poured into an alkaline solution of β -naphthol, triphenyl-1-(2-hydroxynaphthyl)-azophenyl-4-lead was formed. When 2-naphthol-3,6-disulfonic acid or 1-naphthylamine-3,6,8-trisulfonic acid were substituted for β -naphthol, the reaction failed. Diazotized triphenyl-*o*-aminophenyllead and β -naphthol gave triphenyl-1-(2-hydroxynaphthyl)-azophenyl-2-lead.

When various diazonium salts were coupled with triphenyl-*o*-hydroxyphenyllead, the coupling was carried out in a medium composed of 20 ml. of ethyl acetate, 20 ml. of ethyl alcohol, and 40 ml. of 10 per cent sodium hydroxide solution.

p-Nitrobenzenediazonium chloride and triphenyl-*o*-hydroxyphenyllead gave triphenyl-1-[2-hydroxy-3,5-di(*p*-nitrophenylazo)-phenyl]-lead. Triphenyl-*o*-hydroxyphenyllead with *p*-chloro-, *p*-bromo-, *p*-iodo-, and *p*-carboxybenzenediazonium chlorides, respectively, yielded triphenyl-1-[2-hydroxy-5-(*p*-chlorophenylazo)-phenyl]-lead and the corresponding bromo, iodo, and carboxy compounds, respectively. *p*-Sulfonobenzenediazonium chloride did not couple with triphenyl-*o*-hydroxyphenyllead. The latter compound and benzidinetetrazonium chloride gave *p,p'*-biphenylenedi-(5-azo-2-hydroxyphenyltriphenyllead).

The coupling of diazonium salts with triphenyl-*o*-dimethylaminophenyllead was carried out in a reaction medium consisting of 75 ml. of water, 75 ml. of ethanol, 50 ml. of ethyl acetate and 3 g. of sodium acetate. Triphenyl-*o*-dimethylaminophenyllead coupled with *p*-nitro-, *p*-chloro-, *p*-bromo-, *p*-iodo-, and *p*-carboxybenzenediazonium chlorides, respectively, to give triphenyl-1-[2-dimethylamino-5-(*p*-nitrophenylazo)-phenyl]-lead, and the corresponding chloro, bromo, iodo, and carboxy compounds. *p*-Sulfonobenzenediazonium chloride did not couple to any appreciable extent with *o*-dimethylaminophenyltriphenyllead. Triphenyl-1-[4-methoxy-5-(*p*-nitrophenylazo)-phenyl]-lead and triphenyl-1-[2-methoxy-5-(*p*-nitrophenylazo)-phenyl]-lead were obtained by coupling *p*-nitrobenzenediazonium chloride with triphenyl-*p*-anisyllead and triphenyl-*o*-anisyllead, respectively.

When *p*-nitrobenzenediazonium chloride was allowed to react with triethyl-*o*-dimethylaminophenyllead, the resulting products were triethyllead chloride and *p*-nitro-*p'*-dimethylaminophenylazobenzene.

In general, the dyes did not have characteristic melting points. The purity of the products was determined by quantitative analysis for nitrogen and lead. The dyes varied in color from dull red to dark brown.

The structure of each dye was proved by reductive cleavage with stannous chloride and concentrated hydrochloric acid. In each case the compound involved directly in the position of coupling was isolated and identified by a mixed melting point with an authentic specimen.

INTERMEDIARY CARBOHYDRATE METABOLISM OF *ESCHERICHIA COLI*¹

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The dissimilation of glucose by bacteria occurs through a series of phosphorylated intermediates according to the Embden-Meyerhof-Parnas scheme proposed for muscle and yeasts. Glucose is phosphorylated to hexosediphosphate by phosphate contributed from the adenylic acid carrier system. The six-carbon phosphorylated compound is split into two three-carbon phosphate compounds. One of these, phosphoglyceraldehyde, is oxidized to 1,3-diphosphoglyceric acid which is further converted to 3-phosphoglyceric acid by removal of one of the phosphate groups *via* the adenylic acid system. The 3-phosphoglyceric acid is converted to phosphopyruvate by means of an intramolecular shift of the phosphate group and a dehydration. The phosphopyruvate yields its phosphate group to adenylic acid, and the resulting pyruvate is further dissimilated to products determined by the cells or tissue employed. Pyruvate is reduced to lactate in muscle; it is decarboxylated to acetaldehyde which is reduced to ethyl alcohol in yeast; and pyruvate may be reduced, decarboxylated, oxidized to acetic acid and CO₂, converted by condensation to acetylmethylcarbinol and CO₂, or may undergo still other transformations in bacteria.

Almost all of the above evidence was obtained by the use of cell-free preparations of muscle and yeast. Because of technical difficulties the use of whole cells has not been entirely successful. Certain evidence was obtained by use of entire cell preparations of bacteria. This evidence pointed to the existence in bacteria of a system of phosphorylated intermediates, but complete application of the Meyerhof scheme was precluded by lack of suitable cell-free preparations. However, recently two cell-free bacterial preparations became available. Booth and Green (1938) developed a stainless steel roller mill through which a thick bacterial paste circulates. After several hours, a cell-free enzyme system can be obtained by centrifugation. Wiggert *et al* (1940) obtained an active juice from bacteria by mixing a bacterial paste with very finely powdered glass and grinding with a mortar and pestle. Later, a mechanical grinding procedure superseded the earlier method.

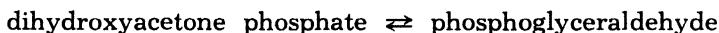
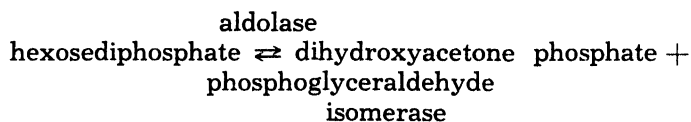
An active preparation was obtained from *Escherichia coli* by means of the glass-grinding method. In this report several of the enzyme systems of bacteria involved in the Embden-Meyerhof-Parnas scheme were compared with the corresponding enzymes of muscle and yeast.

The bacterial preparation shows considerable activity on glucose or a mixture of glucose and hexosediphosphate, when tested on the Barcroft-

¹ Original thesis submitted December 12, 1942. Doctoral thesis number 700.

Warburg respirometer in the presence of NaHCO_3 under an atmosphere of N_2 and CO_2 (10 per cent). The activity is largely due to the production of acid. Extremely active juices will attack hexosemonophosphate and pyruvate, but in general the activity is limited to the conversion of hexosediphosphate to phosphopyruvate and the transfer of phosphate from the latter compound to glucose. The preparation has negligible aerobic activity. Juices prepared from cells grown on a medium containing yeast extract are far more active than juices prepared from cells grown on a peptone medium. The juice contains considerable numbers of viable cells, but the activity of the juice has been shown to be independent of these cells.

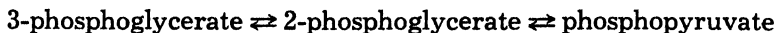
The enzymes involved in the aldolase-isomerase equilibria were first investigated. The reactions are:



A. juice which had been dialyzed for 9 to 12 hours proved suitable for the study of these enzymes. Dialysis removes coenzyme I which catalyzes the oxidation reaction that follows the isomerase equilibrium. The constant of the equilibria was studied with respect to changes in temperature and concentration of enzyme and substrate. Only changes of temperature had any effect. The temperature range of the system is approximately 0 to 50° C. at pH 9, and the pH range is approximately 5.5 to 10.5. The foregoing characteristics of the bacterial aldolase-isomerase enzymes correspond very closely to those of the muscle and yeast enzymes (Meyerhof and Lohmann, 1934).

The aldolase-isomerase equilibria were shown to be truly reversible by the use of triose phosphate as well as hexosediphosphate as a substrate. The triose phosphate was isolated as the bisulfite addition compound. Iodine oxidation of the isolated compound showed that it contained 55-70 per cent phosphoglyceraldehyde as compared with less than 5 per cent of that compound in the normal equilibria mixture. Isomerase is prevented from acting when bisulfite is added to react with the triose phosphate.

The study of the bacterial system was continued with the phosphoglyceromutase-enolase enzymes:



It is possible to study these reactions in the undialyzed juice since phosphopyruvate is not further transformed in the absence of phosphate acceptors. The equilibria constants of the reactions were studied with respect to temperature, and concentration of enzyme and of substrate. Only changes in temperature affect the K_{eq} as in the case of the aldolase-

isomerase equilibria. The temperature range of the system is 0 to 60° C. and the pH range is approximately 6.0 to 10.5.

The equilibria were shown to be truly reversible by the use of both phosphopyruvate and phosphoglycerate as substrates.

The production of phosphopyruvate from phosphoglycerate is stimulated by MgCl_2 or MnSO_4 in a dialyzed juice. The metal ions merely facilitate the establishment of the equilibria without changing the final position. K^+ and Na^+ fail to affect the reaction and Ca^{++} and Ni^{++} are inhibitory. The optimal concentration of Mg^{++} or Mn^{++} is approximately 0.005 M. The metals also stimulate the production of phosphopyruvate in Lebedev juice prepared from dried yeast.

Enolase was shown to be the specific enzyme affected by the metal ions by use of phosphopyruvate as the substrate. The addition of Mg^{++} to the dialyzed enzyme hastened establishment of the equilibrium between 2-phosphoglycerate and phosphopyruvate but inhibited the further conversion of 2-phosphoglycerate to 3-phosphoglycerate.

Apparently, the inhibition of enolase by NaF is caused by the tying-up of the metal component of the enzyme in a metal-fluoride complex. Mn^{++} stimulation of the metal-depleted enzyme is much less susceptible to NaF than is Mg^{++} stimulation.

Phosphopyruvate is not further dissimilated in the juice in the absence of phosphate acceptors. When adenylic acid was added, however, phosphate disappeared from the phosphopyruvate fraction and reappeared in the adenosine triphosphate fraction. The phosphopyruvate was of two types: biologically formed from phosphoglycerate and synthetic. Mg^{++} or Mn^{++} stimulated the transfer in dialyzed juice. As with enolase Mg^{++} stimulation was reversed by NaF while Mn^{++} stimulation was very slightly affected. Phosphate was transferred to glucose from phosphopyruvate when the former was added. Hexosemonophosphate was unsuccessfully substituted as a phosphate acceptor.

Juices prepared with less centrifugation contained the enzyme necessary for the conversion of adenosine triphosphate to adenylic acid and inorganic phosphate (adenosine polyphosphatase). When dialyzed this enzyme was greatly stimulated by the addition of Mg^{++} or Mn^{++} . NaF proved inhibitory in all cases.

All the evidence obtained points to the similarity of the bacterial, yeast, and muscle enzymes. It appears that glucose is dissimilated in the cell-free system obtained from *E. coli* in accordance with the Embden-Meyerhof-Parnas scheme. Application of the system to the entire cell will require further investigation.

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DERIVATIVES OF 2- AND 2,8-SUBSTITUTED DIBENZOFURANS¹

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Orientation studies which have been carried out on dibenzofuran compounds have shown that 2- and 2,8-substituted dibenzofurans can readily be prepared by direct substitution reactions such as halogenation, sulfonation, and the Friedel-Crafts reaction, as well as by ring closures. The preparation of 3,7-substituted dibenzofurans can also be accomplished by direct substitution reactions on dibenzofuran. However, at the present time, metalation by reactive organometallic compounds such as benzylsodium or *n*-butyllithium offers the only convenient, direct method of obtaining 4- and 4,6-substituted dibenzofurans. Metalation has been developed into a very satisfactory way to obtain these compounds. The 1- and 9-positions cannot be attacked by direct nuclear substitution on dibenzofuran itself. The use of 4- and 4,6-substituted dibenzofurans as intermediates in the preparation of 1- and 1,9-substituted dibenzofurans is costly and tedious.

The objectives of this investigation were to prepare 2- and 2,8-substituted dibenzofurans which would make more available 1- and 1,9-substituted dibenzofurans; to clarify the structures of the dibromination products of 2,8-dimethoxydibenzofuran; and to prepare certain derivatives of aminodibenzofurans which might possess pharmacological activity.

The preparation of 2- and 2,8-substituted dibenzofurans, by substitution reactions as well as by ring closures, has been discussed. An attempt has been made to tabulate all of the derivatives of the 2- and 2,8-substituted dibenzofurans which have been reported in the literature prior to January, 1943. The 2- and 2,8-substituted dibenzofurans included in the thesis have also been placed in this table. Derivatives of fused ring systems containing a dibenzofuran nucleus, such as the brazans, dinaphthalene oxides, and the morphine alkaloids have been omitted. In all cases *Chemical Abstracts* has been the criterion in deciding which compounds should be included. The principal references to each compound have been listed. An effort has been made to give a reference to the best preparation of each compound as well as to the report which shows the method used for structural proof.

Several metalation and interconversion reactions were carried out with dibenzofuran and its derivatives. The yield of 4-dibenzofurancarboxylic acid, obtained after carbonation of the organolithium compound, was 2.8 per cent using the relatively unreactive methyllithium for metalation. Metalation of dibenzofuran with phenyllithium gave, after car-

¹ Original thesis submitted March 15, 1943. Doctoral thesis number 712.

bonation, 57 per cent of 4-dibenzofurancarboxylic acid, and *n*-butyllithium gave 76 per cent of the acid under similar conditions.

2-Bromodibenzofuran was metalated to the extent of 39.6 per cent by methyllithium, and 21 per cent by phenyllithium. Interconversion, rather than metalation, occurred when 1,9-(?) -dibromo-2,8-dimethoxydibenzofuran was treated with methyllithium. The yield of 2,8-dimethoxy-1,9(?) -dibenzofurandicarboxylic acid was 54 per cent. 4-Iododibenzofuran gave a 23.6 per cent yield of 4-dibenzofurancarboxylic acid by interconversion with methyllithium, and 37 per cent with *n*-butylmagnesium bromide. 1-Bromo-2-hydroxydibenzofuran and *n*-butyllithium gave, after carbonation, 74 per cent of 2-hydroxy-1-dibenzofurancarboxylic acid.

Lithium did not react with either 2-bromodibenzofuran or 2-iododibenzofuran. Yet, from 4-iododibenzofuran and lithium was obtained, after carbonation, 58 per cent of 4-dibenzofurancarboxylic acid.

The coupling of the Grignard reagents from 2- and 3-bromodibenzofurans proceeded smoothly to yield bi-(2-dibenzofuryl), m.p. 201-202° (25 per cent yield) and bi-(3-dibenzofuryl), m.p. 245-246° (41.8 per cent yield).

2-Aminodibenzofuran was prepared from 2-dibenzofurylmagnesium bromide and α -methylhydroxylamine in 33 per cent yield. In a similar manner 4-aminodibenzofuran was prepared from 4-dibenzofuryllithium and α -methylhydroxylamine in 78 per cent yield.

2-Benzoyldibenzofuran was prepared in three ways. The reaction of 2-dibenzofuryllithium with benzonitrile gave a 36.4 per cent yield. The Friedel-Crafts reaction with benzoyl chloride and dibenzofuran gave a 30 per cent yield. A small amount was obtained from the metalation of 2-dibenzofurancarboxylic acid diethylamide. The purified 2-benzoyldibenzofuran melted at 135-136°, whereas Borsche and Bothe² reported 167-168°. The 2-benzoyldibenzofuran oxime melted at 182-183° instead of 234-235°.²

2,8-Diacetoxydibenzofuran, as well as 2-nitro-3-acetaminodibenzofuran, did not react with bromine in glacial acetic acid.

2-Dibenzofurancarboxylic acid diethylamide, m.p. 77-78°, was prepared in 60 per cent yield from 2-dibenzofurancarboxylic acid chloride and diethylamine. 4-Dibenzofurancarboxylic acid dimethylamide, m.p. 116.5, was prepared in 73 per cent yield from 4-dibenzofurancarboxylic acid chloride and dimethylamine.

Metalation of 2-dibenzofurancarboxylic acid diethylamide with *n*-butyllithium gave, after carbonation, 38.5 per cent of 2-benzoyl-x-dibenzofurancarboxylic acid, m.p. 265-266°; methyl ester, m.p. 189-190°.

Nitration of 2,8-diacetaminodibenzofuran yielded 2,8-diacetamino-3-nitrodibenzofuran, m.p. 322-324°, in 8.9 per cent yield. Hydrolysis with hydrochloric acid gave 76 per cent of 2,8-diamino-3-nitrodibenzofuran, m.p. 210-213°. Deamination gave 3-nitrodibenzofuran (mixed melting point).

² Borsche and Bothe, *Ber.*, 41, 1940 (1908).

Following the procedure of Kirkpatrick and Parker,³ 2-(β -hydroxyethyl)-dibenzofuran was prepared from 2-dibenzofurylmagnesium bromide and ethylene oxide. In addition 1.5 per cent of bi-(2-dibenzofuryl) was obtained.

By means of dry hydrogen bromide the 2-(β -hydroxyethyl)-dibenzofuran was converted into 2-(β -bromoethyl)-dibenzofuran.³ From this compound, by means of the Gabriel synthesis, was obtained 2-(β -aminoethyl)-dibenzofuran.³ The reaction of this compound with benzoyl chloride and sodium hydroxide yielded 78 per cent of N-benzoyl 2-(β -aminoethyl)-dibenzofuran, m.p. 183.5-183.9°. Several attempts to ring-close this compound were unsuccessful.

Following the procedure of Erdtman,⁴ toluhydroquinol dimethyl ether was iodinated to give 5-iodotoluhydroquinol dimethyl ether. This compound was coupled with copper powder to yield 2,2',5,5'-tetramethoxy-4,4'-dimethylbiphenyl.⁴ The next step was a ring closure using hydrobromic acid in glacial acetic acid. The 2,8-dihydroxy-3,7-dimethyldibenzofuran was found to be identical with the tentatively designated 2,8-dihydroxy-3,7-dimethyldibenzofuran prepared by Swislowsky.

Several derivatives of aminodibenzofurans were prepared. By condensation of 3-aminodibenzofuran and *p*-acetaminobenzenesulfonyl chloride was obtained 39 per cent of 3-N²-acetylsulfanilamidodibenzofuran, m.p. 223-224°. On hydrolysis with hydrochloric acid there was obtained 65 per cent of 3-sulfanilamidodibenzofuran, m.p. 245°. This compound was too insoluble to be tested pharmacologically. From 4-aminodibenzofuran and *p*-acetaminobenzenesulfonyl chloride was obtained 26.5 per cent of 4-N²-acetylsulfanilamidodibenzofuran, m.p. 218°. On hydrolysis with hydrochloric acid was obtained 73 per cent of 4-sulfanilamidodibenzofuran, m.p. 195°; which was also too insoluble to be tested.

Condensation of 4-aminodibenzofuran and diethyl bromoethylmalonate yielded 76.6 per cent of diethyl 4-aminodibenzofuran-N-ethylmalonate, m.p. 75-76°. A similar reaction with 3-aminodibenzofuran and diethyl bromoethylmalonate yielded 51 per cent of diethyl 3-aminodibenzofuran-N-ethylmalonate, m.p. 99-100°. 2-Nitro-3-aminodibenzofuran and diethyl bromoethylmalonate did not react.

An attempt was made to reduce 2,8-diamino-3-nitrodibenzofuran to 2,3,8-triaminodibenzofuran by catalytic hydrogenation, but the triamine was too unstable to be isolated in a pure state. The reaction of benzenediazonium chloride with 2,8-dihydroxydibenzofuran gave 1,9-(?)-bisphenylazo-2,8-dihydroxydibenzofuran, m.p. 155-156°, as brick red needles. All attempts to methylate this compound failed.

Several attempts to carry out a Fries rearrangement on 2,8-diacetoxydibenzofuran were failures. Only 2,8-dihydroxydibenzofuran was isolated.

³ Kirkpatrick and Parker, *Jour. Am. Chem. Soc.*, 57, 1123 (1935).

⁴ Erdtman, *Proc. Roy. Soc. London*, A143, 223 (1933).

Treatment of 2-hydroxy-1-dibenzofurancarboxylic acid with acetic anhydride and sulfuric acid yielded 74.5 per cent of 2-acetoxy-1-dibenzofurancarboxylic acid, m.p. 151-152°.

2,6-Dimethylphenol was methylated, then the 2,6-dimethylanisole thus obtained was metalated with *n*-butyllithium. There was obtained 6.7 per cent of 2-methoxy-3-methylphenylacetic acid.

Interconversion of 3-iododiphenyl ether with *n*-butyllithium gave a 50 per cent yield of *m*-phenoxybenzoic acid.

THE COCCIDIA OF WILD RABBITS OF IOWA

I. TAXONOMY AND HOST-SPECIFICITY¹

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INTRODUCTION AND HISTORY

This work was undertaken mainly with the object of clearing up problems of species and host-specificity of the various kinds of coccidia occurring naturally in Iowa wild rabbits. The lack of experimental data, the brevity, and the inaccuracy characterizing past reports on *Eimeria* occurring in this host-group render specific determinations both difficult and precarious.

The same is not so true regarding the *Eimeria* species of the tame rabbit, which were studied with great accuracy by Perard (1924-25) and Kessel and Jankiewicz (1931). The general tendency in the past has been to accept the species found in wild rabbits as the same as those known to occur in tame rabbits. Accurate tests, principally through attempted cross-infections in young rabbits previously free from coccidia, now show that such an assumption has in many instances led to false conclusions.

In the author's opinion, isolated lines of doubtful species should be carefully studied with the aid of cross-infection experiments, since this procedure constitutes a valuable aid in establishing the identity of the coccidia in wild rabbits, their host-specificity, and possible biometrical or morphological changes due to host-environment. The use of young rabbits free from coccidia is a method which should be used henceforth in similar experiments. Old rabbits may show age resistance or even total immunity to certain species.

Only two reports of successful cross-infection between tame and wild rabbits have been reported. Becker (1933) was able to infect and produce clinical coccidiosis in an Iowa cottontail with *Eimeria magna*

¹ Journal paper No. J-1074 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 570. The Fish and Wildlife Service (U. S. Dept. of the Interior), Iowa State College, Iowa State Conservation Commission, and American Wildlife Institute cooperating. Taken from a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, Doctor of Philosophy. Doctoral thesis No. 689. Part I.

These studies were undertaken under suggestion and orientation of Dr. E. R. Becker, to whom the author wishes to express his best thanks for valuable criticism and continuous guidance throughout the work. This work was made possible only with his friendly help and assistance.

Grateful acknowledgments are also extended to Dr. C. J. Drake, Head of the Department, for innumerable courtesies; to Dr. G. O. Hendrickson for constant interest in the work and assistance in obtaining wild rabbits; to Dr. Reeve M. Bailey and other professors and fellow students who contributed to the realization of the present work.

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from the tame rabbit. More recently Jankiewicz (1941) obtained infection with *E. stiedae* from the tame rabbit in the cottontail *Sylvilagus audubonii valicola*. The author was able not only to confirm Becker's work, but in addition, to grow all the tame rabbit species, except *E. stiedae* (not tried), in the Iowa cottontail. Thus, the finding of tame rabbit species in the latter host is, admittedly, a possibility. Nevertheless, for some reason, natural infections of cottontails by these species must be extremely rare, possibly partly on account of lack of contact between the two species.

On the other hand, with the exception of *Eimeria neoleporis* and *E. media*, none of the species occurring in Iowa cottontails could be grown in the tame rabbit, and none of those of the Iowa jack rabbit completed their development in either tame rabbit or cottontail.

As a result of the present experiments, the phenomenon of physiological species of coccidia has emerged. *Eimeria irresidua* and *Eimeria magna* from tame rabbits and jack rabbits present the same morphology in both hosts, but experimental infections have resulted negatively in all trials. Since no notable morphological differences are apparent, it seems preferable to designate the strains occurring in jack rabbits as merely forms or varieties rather than as distinct species. The three new species erected by the author (1942 and present paper), are based on clear morphological differences as well as host-preference.

For specific differentiation the same criteria as those used by Kessel and Jankiewicz (1931) were employed with very satisfactory results. Biometrical data are useful in separating such closely related species as *E. stiedae* and *E. irresidua* or *E. magna* and *E. media*. This procedure is especially recommended for those not well acquainted with morphological details used in specific differentiation; i.e., size of micropyle, residual bodies, and so forth.

The author hopes that the results herein reported will prove useful for further studies in leporine coccidia. Similar studies will eventually have to be made of the *Eimerias* of rabbits not available in Iowa. For this purpose all species of *Eimeria* known so far from *Leporidae* are being included in this paper and the literature cited.

The first *Eimeria* described from wild rabbits was *E. leporis* Nieschulz, 1923. Nieschulz found it in association with *E. stiedae* from hares (*Lepus europeus*) in Holland. For the first time a cross-infection was attempted but with negative results. After that time several European authors were able to confirm Nieschulz's work and many cases were reported on the presence of *E. leporis* either in *Lepus europeus* or *Lepus timidus*.

Fonseca (1932) described *E. pintoensis* from the Brazilian Hare (*Sylvilagus brasiliensis minensis*), and in the following year described *E. paulistana* from the same host, attempting cross-infection with this species and *E. pintoensis* in the tame rabbit, both unsuccessfully.

Yakimoff, Matchuosky, and Spartansky (1936) described *E. septentrionalis* from *Lepus timidus* in Russia. Madsen (1938) described *E.*

sculpta from the Greenland hare and also reported other types. Honess (1939) erected two new species, *E. environ* and *E. maior*, for oocysts found in the Wyoming cottontail, *Sylvilagus nuttallii grangeri* and Carini (1940) described *E. sylvilagi* from the Brazilian hare, referring also to the presence of *E. perforans* in the same host. Carvalho (1942) described *E. neoleporis* from the Iowa cottontail, and effected its growth in the tame rabbit by cross-infection. This was the first proof of a species from wild rabbits growing in tame rabbits.

Other authors who have dealt with the *Eimeria* of wild rabbits since Nieschulz are as follows: Schikarski (1925), Yakimoff, Polueketoff, and Rastegaieff (1931), Henry (1932), Boughton (1932), Robertson (1933), Matsubayashi (1934), Becker (1934), Feurstein (1935), Schoeners (1936), Morgan and Waller (1940).

It is believed that a somewhat confused situation exists in relation to some of the past reports regarding the coccidia of wild rabbits. Cross-infection experiments and the use of young rabbits free from coccidia probably will prove very useful in the future when dealing with doubtful cases.

MATERIAL AND METHODS

These studies were accomplished with the use of coccidia-free rabbits for the hosts. In order to obtain such young rabbits, special precautions were taken. Does were maintained in special cages with grilled bottoms, over pans, so that pellets would pass through the grills and collect in the pans. Systematic cleaning was performed daily by removing the pans, washing them with boiling water, and drying for 48 hours in a room at about 36°C. Cans for providing food and glassware for water were treated in the same way. All food given was left 48 hours in the warm room. It consisted of alfalfa hay, concentrated mixtures, and oats. No green food was provided. Water was constantly available in small Erlenmeyers with a perforated rubber cork, through which a glass tube was inserted.

The young rabbits were separated from the does when from 10 to 15 days old and maintained in screened wire cages, with grilled bottoms and pellet pans. Precautions taken were the same as for adults. They were kept under daily examination for a period of 10 days, so that any spontaneous infection would have time to show up.

A systematic attempt was made to combat cockroaches, flies, mice, and other potential sources of oocyst transmission.

All species of *Eimeria* used in this work were collected at or in the vicinity of Ames. Cottontails and jack rabbits were shot and the intestinal contents examined. Pellets were also collected in the field, principally during the winter season, when they are easily found. In every case that pellets were taken beyond the home range of a given rabbit, they were considered as belonging to a different individual. To permit sporulation the pellets were macerated in the laboratory and placed in a Petri dish with 3 per cent solution of potassium dichromate, about 2 millimeters

deep, at room temperature. During the winter they were maintained at 30°C. in a warm room. Cultures were kept in the same solution, in a refrigerator. Measurements and morphological studies were made on both sporulated and unsporulated oocysts.

Infections were given according to Becker's technique, which consists of injections of oocysts into the stomach through a rubber catheter. In order to determine the dosages administered, counts were made in a hemocytometer.

Routine examinations were done by both usual fresh water and salt flotation methods. Oocyst washing before sporulation was accomplished by centrifuging three or four times in fresh water.

Cross-infection experiments were performed by passing the cottontail species of *Eimeria* into the tame rabbit, those of the jack rabbit into the tame rabbit and the cottontail, and those of the tame rabbit into the cottontail. No young jack rabbits were available for experimentation.

Results obtained in raising young rabbits free from coccidia demonstrated that the best method of controlling unwanted outbreaks of infection in the laboratory is to eliminate oocysts by heat-induced desiccation. It seems clear that no other prophylactic process yields such good results. If pans and other accessories are changed every 48 hours and placed in a warm room at about 36°C., there is no possibility of normal oocyst sporulation. A few oocysts abnormally sporulated are unable to cause further infection. Through this process spontaneous infections brought to the laboratory are easily eliminated and reinfections are very rare.

THE COCCIDIA OF THE TAME RABBIT, *ORYCTOLAGUS CUNICULUS* (LINNAEUS)

1. *EIMERIA STIEDAE* (LINDEMANN, 1865) LUCET, 1913

Psorospermium oviforme Remack, 1854

Monocystis stiedae Lindemann, 1865

Coccidium cuniculi Rivolta, 1878

Coccidium oviforme Leuckart, 1879

(Pl. I, Fig. 2)

Shape: ovoidal or ellipsoidal, slightly narrower at the micropyle end.

Color: salmon tint to yellowish or reddish orange.

Micropyle: present, distinct, convex, very thin, leaving the oval appearance of the oocyst unbroken.

Oocyst wall: thin, with the same thickness throughout, except in the proximity of micropyle where it is thinner. About 0.75 μ in thickness.

Extra-residual body: Absent.

Intra-residual body: present, oval or spherical, about 8.0 by 6.0 μ in dimension.

Sporulation time: 60 to 75 hours; average 65 hours.

Length: range, 28 to 42 μ ; mean, 37.0 μ ; mode, 37.5 μ .

Breadth: range, 16 to 25 μ ; mean, 20.5 μ ; mode, 20.5 μ .

Shape index: 1.8.

Sporocyst: ovoid, with a bluntly pointed anterior end. Average dimensions 8.5 to 10.0 μ by 17.0 to 18.0 μ .

Prepatent period: 6 to 9 days; average 7 days.

Patent period: range 21 to 30 days; average 24 days.

Localization: epithelial cells of the bile ducts.

Specific diagnosis: (1) localization in the epithelial cells of the bile ducts,

(2) presence of intra-residual body and absence of extra-residual body,

(3) sporocyst with bluntly pointed anterior end.

The following hosts harboring *E. stiedae* in nature have been recorded in the literature: *Onyetolagus cuniculus*, *Lepus timidus*, *L. europeus*, *L. californicus*, and *L. americanus*. The single cross-infection experiment reported is the one by Jankiewicz (1941), who obtained its growth in *Sylvilagus audubonii valicola* in California. So far no reference has been made to its natural occurrence in species of *Sylvilagus*. It was found in Iowa tame rabbits, but never in cottontails. No cross-infection experiments were carried on with this species. The possibility of its growth in Mearns cottontails is not discounted since they are able to harbor all other species occurring in tame rabbits when experimentally infected.

The occurrence of *E. stiedae* in wild rabbits in nature is doubted. References based only upon sporulated oocysts may lead to confusion, and such an assertion should be made only after examination of the contents of the gall bladder.

Geographical distribution: cosmopolitan.

2. *EIMERIA PERFORANS* (LEUCKART, 1879) LUCET, 1913

Coccidium cuniculi Rivolta, 1878

Coccidium perforans Leuckart, 1879

(Pl. I, Fig. 5)

Shape: ellipsoidal to slightly oval. Oocyst equally rounded on both ends.

Color: colorless to slightly pinkish.

Micropyle: absent.

Oocyst wall: thicker than in *E. stiedae*. sometimes irregular in outline.

Extra-residual body: present, distinct, small, oval or spherical, measuring about 3.0 μ .

Intra-residual body: present, small, elongated to roundish.

Sporulation time: 30 to 55 hours; average 45 hours.

Length: range, 15.0 to 30.0 μ ; mean 21.5 μ ; mode 23.0 μ .

Breadth: range, 11.0 to 20.0 μ ; mean 15.5 μ ; mode 14.2 μ .

Shape index: 1.4.

Sporocyst: ovoid, with bluntly pointed anterior end. Average dimensions 9.0 to 5.5 μ by 3.5 to 5.0 μ ; mean 8.0 μ by 4.0 μ .

Sporozoite: as usual, with small refractive granules and central nucleus.

Prepatent period: 4 to 5 days.

Patent period: 12 to 24 days.

Intestinal localization: small intestine.

Specific diagnosis: (1) absence of micropyle,
(2) presence of extra- and intra-residual bodies,
(3) small size.

Besides its usual host, *O. cuniculus*, this species has also been reported from *L. americanus*, *L. californicus*, *L. articus*, *S. floridanus mearnsii* and *S. brasiliensis*. The author never found this species occurring naturally in cottontails, as reported by Morgan and Waller (1940), who possibly mistook it for *E. environ*. Its natural occurrence in cottontails is possible, but even then probably in only a very small percentage of them. In a total of nine tame rabbits experimentally infected, the prepatent period was from 4 to 5 days and the patent period from 12 to 24 days.

Geographical distribution: cosmopolitan.

3. *EIMERIA MAGNA* PÉRARD, 1925

(Pl. I, Fig. 1)

Shape: ovoid, broad.

Color: orange-yellow or brownish.

Micropyle: present, very broad and distinct. Usually the margins of the micropyle show peculiar irregularities.

Oocyst wall: with same thickness throughout, except near the micropyle end, where there is a sudden increase in thickness and even formation of hills around the micropyle.

Extra-residual body: present, large, about the same size as the sporocyst, measuring about 9.5 μ in diameter.

Intra-residual body: present, from 1 to 3 μ , sometimes only granular and scattered, but usually oval.

Sporulation time: 48 to 55 hours; average 50 hours.

Length: range, 28 to 42 μ ; mean 35.0 μ ; mode 36.0 μ .

Breadth: range, 20 to 26.0 μ ; mean 24.0 μ ; mode 24.7 μ .

Shape index: 1.4.

Sporocyst: ovoid, without pointed anterior extremity; measuring on the average 11.0 to 16.0 μ by 6.0 to 8.5 μ .

Sporozoite: as usual with large refractive granules and central nucleus.

Prepatent period: 6 to 7 days; average 7 days.

Patent period: 12 to 21 days; average 16 days.

Intestinal localization: lower small intestine, cecum and large intestine.

Specific diagnosis: (1) presence of a wide micropyle,
(2) presence of intra- and extra-residual bodies,
(3) sporocyst with roundish anterior end.

Since Perard's work (1925), *E. magna* has been reported from the following hosts: *O. cuniculus*, *L. europeus*, *L. californicus*, *L. timidus* and *S. floridanus mearnsii*. During these experiments, however, it was never found in the last host.

The prepatent and patent periods taken from fifteen tame rabbits

were found to be, respectively, 6 to 7 and 12 to 21 days; on the average, 7 and 16 days.

The species mentioned by Madsen (1938) as *E. magna* var. *robertsoni* is considered by the author to be good, and is described in more detail in the present paper.

Geographical distribution: cosmopolitan.

4. *EIMERIA MEDIA* KESSEL, 1929

(Pl. I, Fig. 4)

Shape: ovoid.

Color: pinkish to dark orange pink.

Micropyle: present, distinct, convex, more prominent and extending farther back than in *E. stiedae*.

Oocyst wall: with the same thickness throughout, becoming thinner near the micropyle.

Extra-residual body: present, distinct, small, measuring about 5.2 μ .

Intra-residual body: present, small, oval, sometimes scattered and granular.

Sporulation time: 42 to 58 hours; average 52 hours.

Length: range, 27.0 to 36.0 μ ; mean, 31.0 μ ; mode, 31.5 μ .

Breadth: range, 15.0 to 22.0 μ ; mean, 18.5 μ ; mode, 19.0 μ .

Shape index: 1.6.

Sporocyst: very similar to *E. stiedae*, with bluntly pointed anterior end; measuring in average 7.0 by 17.5 μ .

Sporozoite: showing medium-sized refractive granules and central nucleus.

Prepatent period: 6 to 7 days.

Patent period: 15 to 18 days.

Intestinal localization: small intestine.

Specific diagnosis: similar in morphology to *E. magna*, but differs by

(1) smaller size,

(2) small extra- and intra-residual body, as well as micropyle,

(3) oocyst wall thinner near micropyle.

Described by Kessel (1929) from tame rabbits, this species has also been reported from *L. californicus* and a variety of it from *S. nuttallii grangeri*. Only once was it found infecting the cottontail (*S. floridanus mearnsii*) in nature.

The prepatent and patent periods in twelve tame rabbits were, respectively, 6 to 7 and 15 to 18 days. *E. media* may be mistaken for *E. sylvilagi* from cottontails, due to the similarity in size and morphology of the two species. The extra-residual body, however, constitutes a good means of differentiation. Oocysts showing lappets on the sides of the micropyle were seen, suggesting *E. magna*, but in this case, size of oocysts, extra-residual bodies, and sporocysts are useful in differentiating them.

Geographical distribution: reported up to date only from the United States, but probably cosmopolitan.

5. *EIMERIA IRRESIDUA* KESSEL AND JANKIEWICZ, 1931

(Pl. I, Fig. 3)

Shape: ovoid, more blunt at the micropyle end.

Color: light yellow to dark yellow.

Micropyle: present, distinct, concave, sharply delineated.

Oocyst wall: becoming noticeably thicker toward the micropyle end, tapering toward the opposite end, but without the peculiar hills as in *E. magna*.

Extra-residual body: absent.

Intra-residual body: present, distinct, granular, usually very large and elongate.

Sporulation time: 50 to 70 hours; average, 65 hours.

Length: range, 31.0 to 43.0 μ ; mean, 38.3 μ ; mode, 39.4 μ .

Breadth: range, 22.0 to 27.0 μ ; mean, 25.6 μ ; media, 26.5 μ .

Shape index: 1.5.

Sporocyst: large, with a typical sharply pointed anterior end. Average dimensions 10.0 by 20.0 μ .

Sporozoite: with very large refractive granules and central nucleus.

Prepatent period: 9 days.

Patent period: 17 to 21 days.

Intestinal localization: duodenum and jejunum.

Specific diagnosis: (1) lack of extra-residual body,
(2) presence of sharply pointed sporocyst,
(3) shape a characteristic of oocyst wall.

Recently described by Kessel and Jankiewicz (1931), from the tame rabbit, it has also been reported from *L. californicus* and *S. floridanus mearnsii*. The author was unable to find it occurring naturally in the latter host. Oocysts of a similar species were found in the Iowa jack rabbit (*L. campestris townsendii*) and are being described in the present paper.

Geographical distribution: reported up to date only from the United States, but probably cosmopolitan.

6. *EIMERIA EXIGUA* YAKIMOFF, 1934*

(Pl. I, Fig. 6)

Shape: round or ovoid.

Color: colorless.

Micropyle: absent.

Oocyst wall: with uniform thickness.

Extra- and intra-residual bodies: absent.

Sporulation time: unknown.

Length: range, 12.0 to 24.0 μ ; mean, 15.7 μ ; mode, 16.0 μ .

Breadth: range, 9.0 to 16.0 μ ; mean, 13.0 μ ; mode, 12.0 μ .

Mean shape index: 1.25.

Sporocyst: small, about 8.0 to 10.0 μ by 4.0 to 6.0 μ .

* Data taken from original description.

Sporozoite: with central nucleus and no refractive granules (according to Yakimoff's figure).

Prepatent and patent periods: unknown.

Intestinal localization: unknown.

Specific diagnosis: (1) absence of micropyle,
(2) absence of both residual bodies,
(3) small size.

This species described from Russian rabbits was not found to occur in Iowa. Indications are that past reports of this species in wild rabbits, *Sylvilagus* species, are more properly referred to *E. minima* (present paper).

Geographical distribution: Europe (Russia).

THE COCCIDIA OF THE MEARN'S COTTONTAIL, *SYLVILAGUS FLORIDANUS MEARNII* (ALLEN)

1. *EIMERIA ENVIRON* HONESS, 1939

(Pl. I, Fig. 11)

Shape: ovoid to broadly ellipsoidal.

Color: most oocysts colorless, but a few straw-tinged to pinkish.

Micropyle: present, distinct in majority of cases, with a protusible cap, about 5 to 6 μ wide.

Oocyst wall: with uniform thickness, smooth.

Extra-residual body: absent.

Intra-residual body: absent.

Sporulation time: 48 to 75 hours; average 60 hours.

Length: range, 20.0 to 32.5 μ ; mean, 25.7 μ ; mode 25.7 μ .

Breadth: range, 14.3 to 22.8 μ ; mean, 18.5 μ ; mode, 17.5 μ .

Mean shape index: 1.38.

Sporocyst: ovoid, proportionately large, filling most of the space within the oocyst; Stieda's body not seen; 13.0 to 18.5 μ by 7.0 to 9.5 μ .

Sporozoite: comma-shaped, with central nucleus and very large refractive granules (about 6 μ in size).

Prepatent period: 6 days.

Patent period: 15 to 18 days.

Intestinal localization: small intestine.

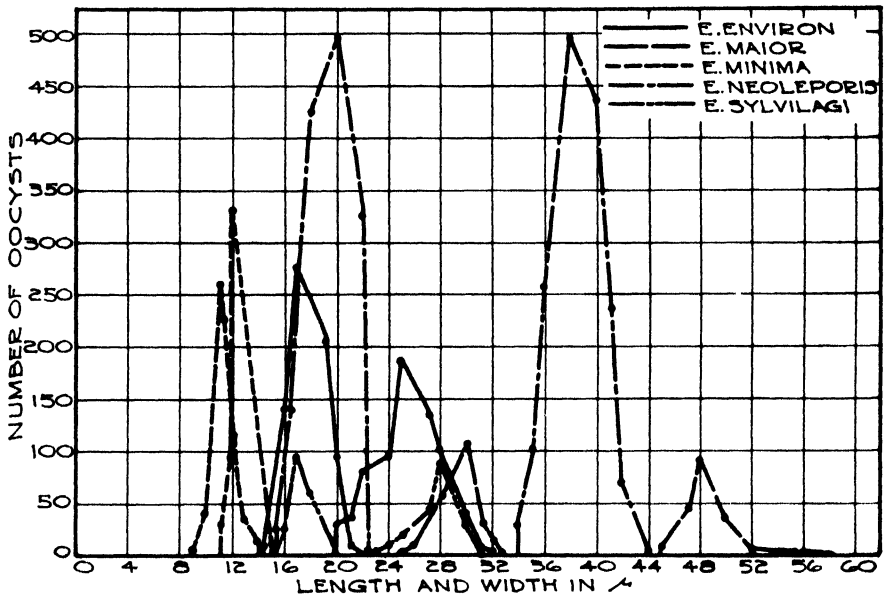
Specific diagnosis: (1) specific for *Sylvilagus*,
(2) lack of intra- and extra-residual bodies,
(3) presence of a micropyle with a protruding cap.

This species, described by Honess (1939) from *Sylvilagus nuttallii grangeri* (Allen) in Wyoming, is here reported for the first time from the Mearn's cottontail and from Iowa. It was found naturally in 64 per cent of the rabbits examined, and is the commonest species in Iowa cottontails. It is believed that Morgan and Waller (1940) misidentified this species as *E. perforans* since they mention the latter as present in the majority of cottontails examined. These species are of approximately

the same size, but oocysts show good differences. The micropyle is evident in *E. environ*, but not in *E. perforans*.

The seasonal distribution is very regular, even during the winter when infections are generally light. Five young cottontails infected with this species in the laboratory showed loss of weight and appetite, but no diarrhea. Doses up to 80,000 oocysts were not lethal.

Geographical distribution: Iowa and Wyoming.



Graph I. Length and width curves for the species of *Eimeria* from the cottontail, *Sylvilagus floridanus mearnsii* (Allen).

2. *EIMERIA MAIOR* HONESS, 1939

(Pl. I, Fig. 7)

Shape: ovoid, slightly tapering toward the micropyle end.

Color: yellowish brown to dark brown.

Micropyle: small, sunken into the oocyst as an identification of the wall; about 8.5 μ .

Oocyst wall: possessing striae which give to it a pectinated appearance. Uneven in thickness, much thinner opposite the micropyle and thickest near the latter. Easily broken under the microscope, showing two distinct layers, the outermost pigmented. Oocyst surface with a sculptured aspect as in fig. 7.

Extra-residual body: present, distinct, varying in shape and size, occasionally dispersed; about 4.2 to 7.1 μ in size.

Intra-residual body: distinct, in a ball, relatively small.

Sporulation time: 70 to 95 hours; average 85 hours.

Length: range, 44.0 to 57.0 μ ; mean, 48.4 μ ; mode, 48.5 μ .

Breadth: range, 25.0 to 35.0 μ ; mean, 29.5 μ ; mode, 30.0 μ .

Mean shape index: 1.60.

Sporocyst: ovoid, in a few cases almost circular; 12.7 to 17.8 μ by 7.0 to 10.1 μ .

Sporozoite: banana-shaped, with central nucleus and small refractive granules (about 2.8 to 4.0 μ in size).

Prepatent period: 6 to 7 days.

Patent period: 9 to 14 days; average 11 days.

Intestinal localization: Unknown.

Specific diagnosis: (1) specificity for *Sylvilagus*,
(2) pectination of the oocyst wall,
(3) large size and superficial sculpture,
(4) presence of extra- and intra-residual bodies.

Originally described from the Wyoming cottontail, this species is here reported for the first time from the Mearn's cottontail and from Iowa. It was found naturally in 13 per cent of the rabbits examined. Morgan and Waller's report (1940) of *E. paulistana* Fonseca, is believed to be properly referred to *E. maior*. All specimens examined by the author possessed the extra-residual body and a small micropyle, neither of which characterizes *paulistana*.

E. maior is admissible to the subgenus *Globidium*, as proposed by Reichnow and Carini (1937) to include those species with an unusual size of all developmental stages and with localization in the subepithelial tissue. For the present the recognition of subgenera in *Eimeria* seems unjustified.

Cottontails infected in the laboratory harbored *maior* without any symptomatology.

Geographical distribution: Iowa and Wyoming.

3. *EIMERIA MEDIA* KESSEL, 1929

(Pl. I, Fig. 4)

This species was found twice, only once naturally. In the latter case, verified in the laboratory, it is presumed that it was due to contact with tame rabbits. Cross-infection experiments were positive, and the oocysts were similar to *E. media* from the tame rabbit in dimensions and characteristics. It is suggested that the case found in nature was probably due to contact of the cottontail with pellets of the tame rabbit.

4. *EIMERIA MEDIA* FORM *HONESSI*, N. FORM

(Pl. I, Fig. 10)

This form, described by Honess (1939) as an unidentified variety of *media*, was found in three of the cottontails examined. Dimensions and characteristics are the same as those given by Honess (1939). One cross-infection experiment was carried on with the tame rabbit, using a light dose of oocysts. No growth was obtained, and the author thinks it advisable for the present to name it as a new form, *honessi*, since that

author did not apply a name to his variety. No further studies were performed and future experiments will determine if it is a good species or not.

5. *EIMERIA NEOLEPORIS* CARVALHO, 1942

(Pl. I, Fig. 8)

Shape: subcylindrical or elongate ellipsoidal, usually tapering somewhat toward the micropyle.

Color: pinkish yellow.

Micropyle: present, very distinct (except in certain perhaps abnormal specimens appearing at the end of heavy infections).

Oocyst wall: smooth, of uniform thickness, noticeably enlarged near the micropyle.

Extra-residual body: consisting of 4 or less granules in the sporoblast stage, or absent, usually disappearing after completion of sporulation.

Intra-residual body: present, large, occupying about $\frac{1}{3}$ of the sporocyst.

Sporulation time: 50 to 75 hours; average 60 hours.

Length: range, 32.8 to 44.3 μ ; mean, 38.8 μ ; mode, 38.5 μ .

Breadth: range, 15.7 to 22.8 μ ; mean, 19.8 μ ; mode, 20.0 μ .

Mean shape index: 1.95.

Sporocyst: with Stieda's body, elliptical, measuring on the average 17.1 μ in length by 8.0 to 9.0 μ in width.

Sporozoite: banana-shaped, with nucleus, large and small refractive granules, about 14.3 to 15.5 μ by 4.0 to 5.0 μ .

Prepatent period: 11 to 14 days; average 12 days.

Patent period: 8 to 16 days; average 10 days.

Intestinal localization: ileo-cecal valve and apical process of the cecum.

Specific diagnosis: (1) presence of a distinct micropyle,

(2) extra-residual body when present only in sporoblast stage and composed of a few granules,

(3) infectivity to tame rabbits,

(4) intestinal localization.

This species, described by the author (1942), was found to occur in 26 per cent of the cottontails examined. Morgan and Waller's report (1940) of *E. leporis* Nieschulz is probably referable to this species. They are closely related, but *neoleporis* differs from *leporis* in its intestinal localization, the presence of a distinct micropyle, the lack of an extra-residual body (or only traces of it during sporoblast stage) and infectivity to tame rabbits.

Cross-infection was positive for tame rabbits and will be discussed later. Large numbers of the oocysts obtained at the end of infections showed the protoplasmic contents diffused throughout, an almost imperceptible micropyle and inability to sporulate. These oocysts were probably unfertilized and bacterial accumulation within them was frequently observed. This contrasts with infections in cottontails where oocysts are always passed in the normal condition, even at the end of

infection. Other than this, no characteristic was present to demonstrate differences of oocysts in the two hosts.

Geographical distribution: Iowa.

6. *EIMERIA SYLVILAGI* CARINI, 1940

(Pl. I, Fig. 9)

Shape: ovoidal to ellipsoidal.

Color: slightly yellowish with a pinkish tinge.

Micropyle: distinct, wide, about 6 μ wide.

Oocyst wall: with uniform thickness (Carini mentioned transverse striae which were not seen by the author). The wall is relatively thick.

Extra-residual body: absent.

Intra-residual body: present, formed by granules scattered among or over the sporozoites.

Sporulation time: 55 to 80 hours; average 65 hours.

Length: range, 22.5 to 31.4 μ ; mean, 29.0 μ ; mode, 28.5 μ .

Breadth: range, 15.0 to 20.0 μ ; mean, 17.5 μ ; mode, 17.0 μ .

Mean shape index: 1.6.

Sporocyst: ellipsoidal, fairly large, with Stieda's body, about 14.5 by 7.0 μ .

Sporozoite: banana-shaped, with central nucleus and small refractive granules (about 4 μ).

Prepatent period: 7 to 8 days.

Patent period: 14 to 18 days.

Intestinal localization: small intestine.

Specific diagnosis: (1) specific to *Sylvilagus*.

(2) absence of extra-residual body,

(3) presence of a granular intra-residual body.

E. sylvilagi was described by Carini (1940) from the Brazilian hare, *Sylvilagus brasiliensis minensis* (Oldfield), and is here reported for the first time in North America. It is readily mistaken for *E. media*, being distinguished by the lack of the extra-residual body.

No pathological symptoms were observed in rabbits harboring this species.

Geographical distribution: Sao Paulo, Brazil, and Iowa.

7. *EIMERIA MINIMA* N. Sp.

(Pl. I, Fig. 12)

Shape: subcircular.

Color: straw-tinged.

Micropyle: absent.

Oocyst wall: with uniform thickness.

Extra-residual body: absent.

Intra-residual body: present, distinct, granular.

Polar granule of oocyst: present, distinct.

Sporulation time: 140 to 160 hours.

Sporocyst: ovoid, about 2.5 by 5.7 μ .

Sporozoite: as usual, with very small refractive granules.

Length: range, 11.0 to 15.0 μ ; mean, 13.4 μ ; mode, 14.0 μ .

Breadth: range, 9.0 to 14.0 μ ; mean, 10.8 μ ; mode, 11.0 μ .

Mean shape index: 1.2.

Prepatent period: 6 days.

Patent period: 12 to 16 days.

Intestinal localization: small intestine.

Specific diagnosis: (1) specificity for *Sylvilagus*.

(2) presence of intra-residual body,

(3) presence of polar granule.

This small species of *Eimeria* was found to occur in 13 per cent of the cottontails examined by the author. Its small size and unusually light infections render it difficult to find in ordinary examinations. Sporulation was always irregular when in 3 per cent potassium dichromate or 2 per cent formic acid. It was most effective when pellets were macerated, passed through a fine mesh, washed by centrifugation 2 or 3 times, and then placed in fresh water. The presence of the protozoan *Golpoda inflata* constituted a great help in keeping down the bacterial flora. No oocysts were observed within this protozoan. The amount of liquid added should be only enough to keep the contents fairly moist, since oocysts in a layer of liquid 3 mm. thick were always delayed in sporulation. Contrary to expectation, the small oocysts of this species require the longest sporulation time of any species of *Eimeria* found in rabbits and hares.

Cross-infection experiments conducted with two young tame rabbits resulted unsuccessfully. This fact leads the author to believe that past reports on *E. exigua* from the Mearns cottontail are properly referable to this species. The presence of a distinct intra-residual body, a polar granule and non-infectivity to the tame rabbit permit the author to raise it to specific rank under the name *minima*.

Geographical distribution: Iowa.

THE COCCIDIA OF THE WHITE-TAILED JACK RABBIT, *LEPUS TOWNSENDII* CAMPANIUS HOLLISTER

1. *EIMERIA AMERICANA* N. Sp.

(Pl. I, Fig. 14)

Shape: ovoid or elongated ellipsoidal, tapering toward the extremities.
Color: pinkish.

Micropyle: present, distinct, about 6 μ wide.

Oocyst wall: delicate, with uniform thickness. A distinct dark-brownish marginal lappet visible of the micropyle.

Extra-residual body: present, distinct, granular and spread over and among the sporocysts.

Intra-residual body: absent.

Sporulation time: 55 to 65 hours; average 60 hours.

Length: range, 34.3 to 42.8 μ ; mean, 38.1 μ ; mode, 38.8 μ .

Breadth: range, 18.3 to 25.0 μ ; mean, 21.0 μ ; mode, 21.3 μ .

Mean shape index: 1.8.

Sporocyst: ovoid, proportionately large, about 7.0 to 8.0 μ by 17.1 μ .

Sporozoite: comma-shaped, with central nucleus and large refractive granules, about 5.5 μ .

Prepatent, patent period and intestinal localization: unknown.

TABLE 1
DISTRIBUTION OF SPECIES IN INDIVIDUAL WILD COTTONTAILS

Rabbit No.	Date	<i>E. neoleporis</i>	<i>E. enuron</i>	<i>E. maior</i>	<i>E. sylvilagi</i>	<i>E. media</i>	<i>E. minima</i>
1*	9- 8-41		X				X
2*	9-12-41		X	X			
3*	"		X		X		
4	9 20-41						X
5*	"	X	X		X		X
6	9 28 41		X			X	
7	"		X				
8	10 5-41	X	X				
9*	10-12-41		X				
10	11- 5-41						
11*	11-26-41	X	X				
12*	"	X					
13*	"		X				
14*	1 30 42						
15	"		X				
16	2 6-42						
17	"		X				
18	2 20-42				X		
19	"			X			
20	"		X		X		
21	"	X	X				
22*	"	X	X		X		
23	"		X				
24*	"	X	X	X			
25	2-26-42		X				
26	"						X
27*	2-27 42		X				
28*	"	X	X		X		
29	"			X	X		
30	"		X	X			
31	3 2 42				X		
32*	"	X	X				
33	4 5 42		X				
34	"						
35*	4 8-42						
36*	4-20-42	X	X		X		
37*	"		X				
38*	6- 2-42		X				X
39*	"		X				
40*	6-10-42	X	X		X		
41*	6-22-42						
42*	6-25-42			X			X
43	7- 3-42				X		
44	"		X				
45*	7- 5-42	X			X		
Total.....		12	29	6	12	1	6
Percentage.....		26	64	13	26	2	13

* Based on autopsies of field-collected cottontails. All others are based on examination of cultures from field-collected pellets.

- Specific diagnosis:* (1) specific for the jack rabbit,
(2) presence of extra-residual body and absence of intra-residual body,
(3) shape of the oocyst, and
(4) lappet surrounding the micropyle.

This species was found in two jack rabbits. Its peculiar anterior end, with a prominent lappet around the micropyle, make its identification fairly easy. Cross-infection experiments with tame rabbits and cottontails resulted unsuccessfully.

Geographical distribution: Iowa.

2. *EIMERIA IRRESIDUA* FORM *CAMPANIUS* N. FORM.

(Pl. I, Fig. 15)

Oocyst with the same morphology and dimensions of *E. irresidua* from the tame rabbit were recovered from two jack rabbits. Trials of cross-infection with tame rabbits and cottontails, however, were negative. The new physiological form *campanius* is erected for this type.

3. *EIMERIA MAGNA* FORM *TOWNSENDII* N. FORM.

(Pl. I, Fig. 1)

This new form is being erected for oocysts recovered from three jack rabbits. It is morphologically similar to *E. magna* from the tame rabbit, but is unable to grow in either that host or the cottontail. Cross-infections were tried with six young tame rabbits free from coccidia, to which heavy and fresh doses of oocysts were given. Two cottontails which also received heavy doses were not susceptible.

4. *EIMERIA ROBERTSONI* MADSEN, 1938

(Pl. I, Fig. 13)

Shape: ovoid to broad ellipsoidal. Approaching *irresidua* more closely than *magna*.

Color: slightly yellow to pinkish-yellow.

Micropyle: present, distinct, very large; average 8.5 μ .

Oocyst wall: with uniform thickness throughout except near the micropyle where it is thicker and usually with prominent marginal lappet.

Extra-residual body: present, very large, usually 12.0 to 15.0 μ , almost twice the normal size of *magna*.

Intra-residual body: normally absent; if present with the granules few and scarcely visible. Never distinct or in a ball as in *magna*.

Sporulation time: 50 to 65 hours; average 56 hours.

Length: range, 35.7 to 46.8 μ ; mean, 40.5 μ ; mode, 41.8 μ .

Breadth: range, 22.8 to 32.8 μ ; mean, 25.0 μ ; mode, 25.7 μ .

Mean shape index: 1.6.

Sporocyst: oblong-fusiform, with relatively sharp anterior end, about 19.5 by 7.0 μ , some as long as 22.0 μ , which never occurs in *magna*.

Sporozoite: elongate, comma-shaped, with fairly large refractive granules.

Prepatent and patent periods: unknown.

Intestinal localization: duodenum.

Specific diagnosis: (1) not growing in tame rabbits or cottontails.

(2) extra-residual body, size and sporocysts all very large,

(3) lack of intra-residual body or, if present, only traces.

This species is being raised to specific rank, because it differs from *E. magna* in the following characters:

Species	<i>E. magna</i>	<i>E. robertsoni</i>
Size	Mean length and width: 35.0 by 24.0 μ .	Mean length and width: 40.5 by 25.0 μ .
Extra-res. body	Smaller, about 7.5 to 9.0 μ .	Larger, about 12.0 to 15.0 μ .
Micropyle	Smaller, about 6.0 μ .	Larger, about 8.5 μ .
Intra-res. body	Distinct, ovoid, about 1.0 to 3.0 μ .	Absent or indistinct.
Sporocyst	Mean length 14.0 μ .	Mean length 19.5 μ .
Localiza- tion	Lower small intestine, ce- cum, and large intestine.	Duodenum.
Hosts	<i>Oryctolagus cuniculus</i> .	<i>L. arcticus</i> and <i>L. town- sendii</i> .

E. robertsoni occurred in nine of the twelve (75 per cent) jack rabbits examined. Cross-infection trials with tame rabbits and cottontails were negative. The figure presented by Madsen (1938) for his type B (figure b) is probably a representative of this species. He mentioned it under the name of *perforans* var. *groenlandica*, but *perforans* reaches a maximum length of only 30 μ . The micropyle which is very distinct in his picture, is absent in *perforans*. It is probable that Madsen's description is a complex of two species, but for nomenclatorial purposes the name *robertsoni* may be associated with the larger form here described (Fig. 5, b, and Fig. 6, a, of Madsen).

Geographical distribution: Greenland and Iowa.

5. *EIMERIA SEPTENTRIONALIS* YAKIMOFF, MASTCHUOSKY AND SPARTANSKY, 1936

(Pl. I, Fig. 17)

Shape: ovoid to circular.

Color: transparent or light-violet.

Micropyle: present, very broad, measuring up to 12 μ .

Oocyst wall: with uniform thickness of 1 μ throughout; near the micropyle there is a prominent marginal lappet, which is darker in color than the rest of the wall.

Extra-residual body: absent.

Intra-residual body: absent.

Sporulation time: 55 to 65 hours; average 60 hours.

Length: range, 22.8 to 32.0 μ ; mean, 23.8 μ ; mode, 22.8 μ .

Breadth: range, 20.0 to 22.8 μ ; mean, 20.6 μ ; mode, 20.0 μ .

Mean shape index: 1.1.

Sporocyst: ovoid to ellipsoidal, normal size 12.0 to 14.0 μ by 6.0 to 8.0 μ .

Sporozoite: as usual, with central nucleus and medium size refractive granules.

Prepatent and patent periods: unknown.

Intestinal localization: unknown.

Specific diagnosis: (1) non-infectivity to tame rabbits.

(2) very broad micropyle,

(3) lack of intra- and extra-residual bodies.

Described from the Russian hare (*Lepus timidus*), *Eimeria septentrionalis* has been reported also from the Greenland hare, *Lepus arcticus* (by Madsen, 1938). This author reported *Eimeria septentrionalis* under the name *E. exigua* var. *septentrionalis*. *E. exigua* is an entirely distinct species which is smaller and does not possess a micropyle. Madsen's statement, "Micropyle absent or, if present, broad, more or less indistinct, sometimes surrounded by a thickened margin," leads the author to believe that he dealt with two species, or saw and drew specimens with the micropyle turned down on the microscope field (hence invisible).

E. septentrionalis is here being reported for the first time from *Lepus townsendii campanius* and from the United States. It was found in four of the twelve (33 per cent) jack rabbits examined. Cross-infection experiments with tame rabbits and cottontails failed to show any infection. Our specimens were similar to the ones described by Yakimoff *et al.*, and the author regards it as a good species.

Geographical distribution: Europe (Russia), Greenland, and United States (Iowa).

6. *EIMERIA SCULPTA* MADSEN, 1938

(Pl. II, Fig. 16)

Shape: pear-shaped to ovoidal.

Color: Dark-brown.

Micropyle: narrow, plug-shaped (Madsen). In our specimens the micropyle was broad, measuring up to 7.5 μ .

Oocyst wall: with uniform thickness of about $2\ \mu$, but somewhat thicker near the micropyle. Outer surface distinctly sculptured.

Extra-residual body: absent.

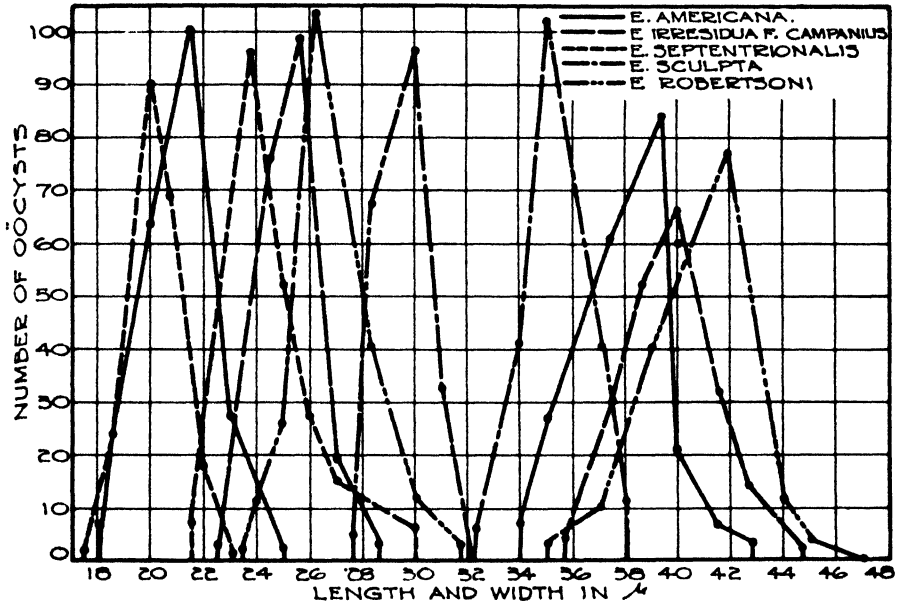
Intra-residual body: large, sharply delimited, ellipsoidal (Madsen). In our specimens granular, coarse, without a fixed form.

Sporulation time: 50 to 60 hours; average 55 hours.

Length: range, 32.0 to 38.0 μ ; mean, 36.0 μ ; mode, 35.7 μ .

Breadth: range, 28.6 to 31.4 μ ; mean, 29.7 μ ; mode, 30.0 μ .

Mean shape index: 1.2.



Graph II. Length and width curves of the species of *Eimeria* from the jack rabbit, *Lepus townsendii campanius* Hollister.

Sporocyst: ovoidal, with sharply pointed end, measuring 15.0 to 19.0 μ by 9.0 to 10.0 μ . No micropyle was seen as mentioned by Madsen.

Sporozoite: as usual, with central nucleus and small refractive granules.

Prepatent and patent periods: unknown.

Intestinal localization: unknown.

Specific diagnosis: (1) not growing in the tame rabbit or cottontail,
(2) sculptured surface,
(3) absence of extra-residual body and presence of intra-residual body.

Described by Madsen (1938) from *Lepus arcticus*, this species is here reported for the first time from *Lepus townsendii campanius* and from the United States. It was found in nine of the twelve (75 per cent) jack rabbits examined.

Cross-infection experiments were negative for tame rabbits and

cottontails. Our specimens possessed a much wider micropyle than Madsen's specimens. The oocyst wall was thicker near the micropyle, and several specimens showed the marginal lappet around it. No other differences were noted.

Geographical distribution: Greenland and Iowa.

TABLE 2
DISTRIBUTION OF SPECIES IN JACK RABBITS

Rabbit	Date	<i>E. robertsoni</i>	<i>E. sculpta</i>	<i>E. septentrionalis</i>	<i>E. americana</i>	<i>E. irresidua f. campanius</i>
1.....	10- 8-41	X		X		
2.....	3-14-42	X	X		X	
3.....	"	X	X		X	
4.....	"	X	X		X	
5.....	3-18-42	X	X	X	X	X
6.....	"	X				
7.....	3-22-42	X	X		X	
8.....	"		X			
9.....	"	X	X			
10.....	4- 2-42			X		
11.....	"	X	X	X		
12.....	"		X			X

THE COCCIDIA OF OTHER RABBITS AND HARES*

1. *EIMERIA LEPORIS* NIESCHULZ, 1923

(Pl. I, Fig. 18)

Shape: strikingly narrow, usually cylindrical, more rarely elliptical and frequently weakly bent into a bean form.

Color: completely colorless or with a very weak tinge of yellow.

Micropyle: absent (according to Nieschulz a clear micropyle could not be recognized).

Oocyst wall: present, of constant size, breaking down into small individual granules after the completion of sporulation.

Intra-residual body: roundish, large.

Extra-residual body: present, of constant size, breaking down into small individual granules after the completion of sporulation.

Sporulation time: unknown.

Length: range, 26.0 to 36.0 μ ; mean, 32.0 μ .

Breadth: range, 13.0 to 20.0 μ ; mean, 16.0 μ .

Mean shape index: 2.0.

Sporocyst: ovoid, with pointed anterior end. Stieda's body present.

Sporozoite: elongate, with large refractive granules.

Prepatent and patent periods: unknown.

Intestinal localization: small intestine.

* Data given for these species were obtained from the original descriptions, since none of them have been examined by the author.

Specific diagnosis: (1) specificity to *Lepus* spp,
(2) absence of micropyle,
(3) presence of large extra-residual body,
(4) intestinal localization.

Cross-infection experiments conducted by Nieschulz have shown that this species does not grow in tame rabbits. The author was unable to detect this species in the Iowa cottontail, as reported by Morgan and Waller (1941). A closely related species was named *E. neoleporis* by the author. A comparison of the figures given by Nieschulz (1923) and Schoeners (1936) for *E. leporis*, and Pl. I, Fig. 8, for *E. neoleporis* illustrates the differences.

Geographical distribution: Europe.

2. *EIMERIA PAULISTANA* FONSECA, 1932

(Pl. I, Fig. 20)

Shape: elliptical-elongate and regular, flattened at the micropyle end.

Color: heavily bile-stained.

Micropyle: present, distinct, very large (as seen in Fonseca's figure).

Oocyst wall: very thick and formed by three membranes; the innermost thin and adherent to the sporoblast, the median thicker, separated from each other and from the outer one. The latter with double contour, striated transversally and thicker near the micropyle.

Extra-residual body: absent.

Intra-residual body: present, small and ovoid (as seen in Fonseca's figure.)

Sporulation time: minimum 120 hours.

Length: range, 40.0 to 43.0 μ ; mode, 43.0 μ .

Breadth: range, 23.5 to 24.0 μ ; mode, 24.0 μ .

Shape index: 1.7.

Sporocyst: ovoid, measuring 15.5 by 7.5 μ .

Sporozoite: elongate.

Prepatent and patent periods: unknown.

Intestinal localization: unknown.

Specific diagnosis: (1) specificity for *Sylvilagus*,
(2) presence of intra-residual body and lack of extra-residual body,
(3) striated wall and large micropyle.

This species described from the Brazilian hare is very closely related to *E. maior* from the American *Sylvilagus*, but according to Fonseca (1933), it lacks an extra-residual body, which is present in the latter. It was mentioned by Morgan and Waller (1941) as occurring in the Iowa cottontail, but was not found in the present investigation. Experimental infections carried through in the cottontail always revealed a distinct extra-residual body in the oocysts, in absolute agreement with Honess' (1939) description for *E. maior*. Oocysts exchanged with the latter author proved to be the same species.

Cross-infection experiments carried out by Fonseca in the tame

rabbit have proved the specificity of this species for *Sylvilagus*, as is the case with *E. maior*.

Geographical distribution: Brazil.

3. *EIMERIA PINTOENSIS* FONSECA, 1932

(Pl. I, Fig. 19)

Shape: ovoid, narrower toward the micropyle end.

Color: faintly yellowish-green.

Micropyle: invisible in immature specimens. Present in mature specimens, but not very distinct.

Extra-residual body: absent.

Intra-residual body: present, elongated.

Sporulation time: 48 hours.

Length: range, 21.5 to 26.5 μ ; mean, 23.5 μ .

Breadth: range, 15.0 to 16.0 μ ; mean, 15.5 μ .

Shape index: 1.5.

Sporocysts: ovoid, usual size 12.0 to 14.0 by 5.0 to 7.0 μ .

Sporozoites: according to Fonseca's figure, as usual, with small refractive granules.

Prepatent and patent periods: Unknown.

Intestinal localization: unknown.

Specific diagnosis: (1) occurring in Brazilian *Sylvilagus*,

(2) absence of extra-residual body and presence of intra-residual body,

(3) indistinct micropyle.

This species was not found in the Iowa cottontail in spite of diligent search. Attempted cross-infection experiments with the tame rabbit were unsuccessful (Fonseca).

Geographical distribution: Brazil.

A KEY FOR THE SPECIES OF *EIMERIA* KNOWN FROM RABBITS AND HARES

1. Micropyle absent 2
Micropyle present 6
2. Extra- and intra-residual bodies present 3
Both residual bodies absent, or only the intra-residual body present 4
3. Small oocyst, not over 30 μ in length, shape index 1.4, normally in tame rabbits (Pl. I, Fig. 5) *E. perforans* (Leuckart)
Large oocyst, over 26 μ in length, shape index 2.0, in European *Lepus* (Pl. II, Fig. 18) *E. leporis* Nieschulz
4. Only the intra-residual body present 5
Both residual bodies absent (Pl. I, Fig. 6) *E. exigua* Yakimoff
5. Small oocyst, not over 15 μ in length, shape index 1.2, in the American *Sylvilagus* (Pl. I, Fig. 12) *E. minima* n. sp.
Large oocyst, over 21 μ in length, shape index 1.5, in the Brazilian *Sylvilagus* (Pl. I, Fig. 19) *E. pintoensis* Fonseca

6. Extra-residual body distinct in sporulated oocysts 7
Extra-residual body absent, or consisting of but 3 or 4 granules during sporoblast stage12
7. Intra-residual body present and distinct 8
Intra-residual body absent or consisting of but 3 or 5 scattered granules10
8. Very large oocyst, with sculptured surface and pectinated wall, growing only in *Sylvilagus* (Pl. I, Fig. 7)*E. maior* Honess
Not as above, growing in *Lepus* or *Oryctolagus* 9
9. Small oocyst, mean length 31.0 μ , shape index 1.6, with small residual body and convex micropyle (Pl. I, Fig. 4)*E. media* Kessel
Medium size to large oocyst with a marginal lappet, shape index 1.4, with very large extra-residual body and concave micropyle (Pl. I, Fig. 1)*E. magna* Perard
Same as above, but specific for *Lepus*
E. magna form *townsendii* n. form
10. Very large oocyst, mean length 40.5 μ , extra-residual body averaging over 12.0 μ , shape index 1.6, oocyst wall thickening toward the micropyle (Pl. I, Fig. 13)*E. robertsoni* Madsen
No so large as above, extra-residual body small or scattered throughout the oocyst, the oocyst wall with uniform thickness.....11
11. Mean length 38.1 μ , shape index 1.8, extra-residual body granular and spread over and among the sporocysts, in *Lepus* (Pl. I, Fig. 14)
E. americana n. sp.
Mean length 28.5 μ , shape index 1.4, extra-residual body small, in *Sylvilagus* (Pl. I, Fig. 10).....*E. media* form *honessi* n. form
12. Intra-residual body present.....13
Intra-residual body absent18
13. Very large, with thick oocyst wall, mean length 43.0 μ , shape index 1.7, in the Brazilian *Sylvilagus* (Pl. I, Fig. 20)....*E. paulistana* Fonseca
Not as above14
14. Elongate, mean length 38.8 μ , shape index 1.95, oocyst tapering toward the anterior end, in the American *Sylvilagus* (Pl. I, Fig. 6)
E. neoleporis Carvalho
Oocyst round or ovoid, shape index smaller than above15
15. Oocyst with sculptured surface, wide micropyle, mean length 36.0 μ , shape index 1.2, in *Lepus* (Pl. I, Fig. 16)*E. sculpta* Madsen
Oocyst not sculptured, occurring normally either in *Sylvilagus* or *Oryctolagus*16
16. Mean length 29.0 μ , shape index 1.6, intra-residual body granular and scattered, in *Sylvilagus* (Pl. I, Fig. 9)*E. sylvilagi* Carini
Mean length over 29.0 μ , in *Oryctolagus*.....17
17. Mean length 37.0 μ , shape index 1.8, convex micropyle, localized in the liver (Pl. I, Fig. 2).....*E. stiedae* (Lindemann)
Mean length 38.3 μ , shape index 1.5, concave micropyle, localized in the intestine (Pl. I, Fig. 3).....*E. irresidua* Kessel & Jankiewicz
Same as above, but growing only in *Lepus*
E. irresidua form *campanius* n. form

18. Subspherical, mean length 32.8 μ , shape index 1.1, with large micropyle, in *Lepus* (Pl. I, Fig. 17) *E. septentrionalis* Yakimoff et al.
Ovoid, mean length 25.7 μ , shape index 1.3, with narrow and plug-shaped micropyle, in *Sylvilagus* (Pl. I, Fig. 11) *E. environ* Honess

EXPERIMENTAL CROSS-INFECTIONS WITH THE SPECIES OF THE TAME RABBIT, COTTONTAIL, AND JACK RABBIT

1. THE SPECIES OF THE TAME RABBIT INTO COTTONTAILS

E. PERFORANS. Infection was obtained by feeding freshly sporulated oocysts of this species to three young cottontails. The oocysts recovered from these cottontails were similar in size and morphology to normal oocysts from tame rabbits which averaged 21.5 μ in length by 15.5 μ in width. Table 3 shows the length and breadth of *E. perforans* in cottontails.

TABLE 3
LENGTH AND BREADTH OF *E. perforans* RECOVERED FROM EXPERIMENTAL INFECTION IN A COTTONTAIL

Length	Breadth					Total
	12.8	14.3	15.7	17.1	18.5	
18.5	1	2	2	1		6
20.0	2	9	5	3		19
21.4	3	16	12	2		33
22.8	9	46	26	1		82
24.3	7	23	16	1	1	48
25.7	2	3	5	1	1	12
Total	24	99	65	9	2	200

No special symptoms were observed, a condition attributed to the small dose given. The prepatent period was 5 days and the patent period from 12 to 24 days or the same as in tame rabbits. One case of natural infection in the laboratory was verified, following placement of a cottontail in a tame rabbit cage.

E. IRRESIDUA. Cottontails proved to be receptive to this species. The prepatent period averaged 9 days, but the patent period was irregular, varying from 8 to 17 days. Oocysts recovered from cottontails were, however, similar to those obtained from tame rabbits which averaged 38.3 μ in length by 25.6 μ in width. Table 4 shows the range in size of the oocysts recovered from the pellets of the cottontails.

E. STIEDAE. No attempts were made to grow this species in Mearn's cottontails. It should probably prove to be infective, however, because all other species from the tame rabbit, in which cross-infection has been attempted, were able to develop in this host.

E. EXIGUA. No oocysts of this species from the tame rabbit were available for cross-infection to cottontails.

E. MEDIA. This species was also able to produce infection in cottontails, but no symptomatology was apparent. The prepatent period was from 5 to 6 days and the patent period from 14 to 16 days. Oocysts recovered from cottontails, as shown in Table 5, and those from tame rabbits were similar in morphology and size. The original tame rabbit strain averaged $30.0\ \mu$ in length by $18.5\ \mu$ in width.

E. MAGNA. As previously reported by Becker (1933), this species is infective to cottontails, producing clinical coccidiosis. From the third

TABLE 4
LENGTH AND BREADTH OF *E. irresidua* RECOVERED FROM EXPERIMENTAL
INFECTION IN A COTTONTAIL

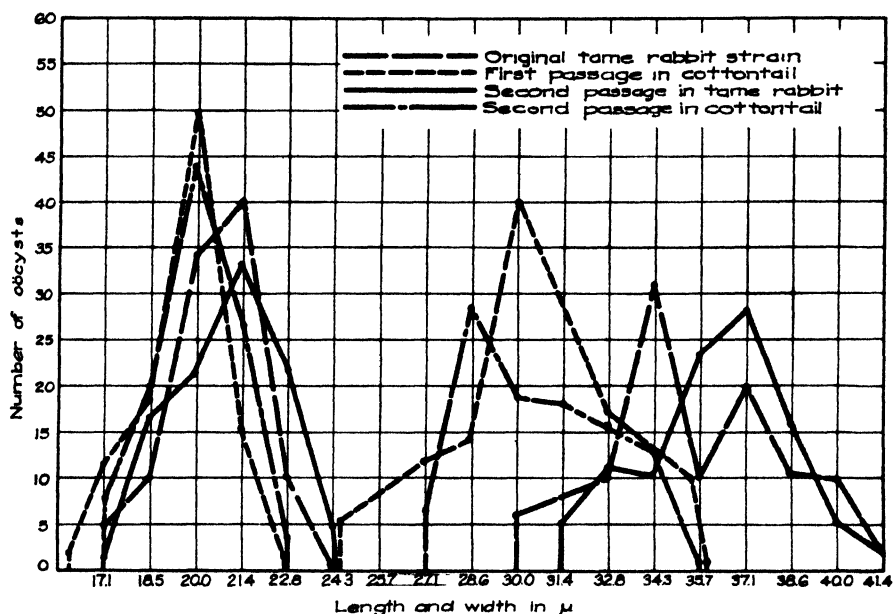
Length	Breadth						Total
	20 0	21 4	22 8	24 3	25 7	27 1	
32 8	1	1	3	4	8	1	18
34 3			4	9	8		21
35 7			4	15	12	2	33
37 1		2	1	7	14		24
38 6			1	4	4	1	10
40 0				1			1
Total	1	3	13	40	46	4	107

to the sixth day, the cottontails showed rough coat, loss of appetite, and softened pellets. The prepatent period averaged 4 days and the patent period 14 days. Tame rabbits infected at the same time with identical dosage and culture had a prepatent period of from 6 to 7 days and a patent period of from 12 to 21 days. There was a difference not only in respect to the duration of the prepatent period, but also in size of oocysts produced in the two species of host. The morphology, however, was constant, and no further modifications occurred in the residual bodies or sporocysts. Graph III compared the size of *E. magna* from cottontails

TABLE 5
LENGTH AND BREADTH OF *E. media* RECOVERED FROM EXPERIMENTAL
INFECTION IN A COTTONTAIL

Length	Breadth						Total
	15.7	17.1	18 6	20.0	21 4	22 8	
24.3		2	1	1	1		5
25.7	3	3	2	1			9
27.1	6	8	4	1			19
28.6	3	19	4	9	1		36
30.0		5	3	1			9
31.4		2	2	4	1		9
Total	12	39	16	17	3		87

and tame rabbits. When the different results are compared for significance, the following data are obtained: In the case of the original strain in the tame rabbit and the first passage in the cottontail, the Diff. means for width was 8.9, and for length, 23.5; the same for P.E.diff.means the second passage in the tame rabbit and second passage in the cottontail was, for width, 5.7, for length, 25.4; for original tame rabbit strain and



Graph III. Comparative size of *E. neoleporis* in tame rabbits and cottontails (based on 100 oocysts).

second passage into the tame rabbit, width 0.6, length 0.3; first passage into cottontail and second passage into cottontail, width 2.1, length 0.9. Since it is usually assumed that a value of 3 is significant, it is obvious that length and width of the oocyst of *E. magna* differed significantly in the tame rabbit and cottontail in both the first and second passages through the two hosts, and that the dimensions did not differ significantly in the two passages through either species.

The data below show the means, standard deviation, coefficient of variation, etc. of the species in the different rabbits.

The minor differences in the curves of the original tame rabbit strain and the second passage in tame rabbit are probably due to normal variation within the strain. This species, however, has a wide length range, much wider than in any other species of the tame rabbit. It is apparent, even in spite of the variable length, that the mode was considerably changed when this species was grown in the cottontail. The normal mode was attained when oocysts recovered from cottontail were passed back

into the tame rabbits, and again modified by a second passage in the cottontail. This is the first time that a significant change in size of the oocysts due to host-environment has been reported, and it may prove of help for future taxonomic studies.

2. THE SPECIES OF THE COTTONTAIL INTO TAME RABBITS

E. ENVIRON. Five young and two adult rabbits which received doses up to 100,000 oocysts of this species, failed to show infection.

E. MAIOR. Three young and two adult tame rabbits were fed doses

TABLE 6
BIOMETRICAL DATA OBTAINED WITH SERIAL PASSAGES OF *E. magna*
INTO TAME RABBITS AND COTTONTAILS

Passages	Original Tame Rabbit Strain	First Passage in Cottontail	Second Passage in Tame Rabbit	Second Passage in Cottontail
Width	20 35 \pm 0 08	19.46 \pm 0 06	20 43 \pm 0 11	19.67 \pm 0.08
σ	1 252	1.388	1 702	1.500
c.v.	6 15 \pm 0 29	7 132 \pm 0 24	8 33 \pm 0.38	7.62 \pm 0.35
Length.	35 57 \pm 0 20	30 09 \pm 0 12	35.65 \pm 0.16	30.26 \pm 0.14
σ	2 97	2 52	2 57	2.26
c.v.	8 35 \pm 0 40	8.40 \pm 0.29	7.20 \pm 0.33	7.26 \pm 0.35
Mean shape index	1.74	1.54	1.74	1.53

up to 50,000 oocysts, and no infection developed.

E. NEOLEPORIS. Several infections were obtained with this species when oocysts were fed to young tame rabbits. The experimental work on this subject will constitute the second article on the coccidia of the wild rabbits of Iowa.

E. MINIMA. Two young rabbits fed with oocysts of this species failed to show infection.

E. MEDIA. Oocysts of the single case of natural parasitism of this species found in the cottontail proved to be infective to the tame rabbit. It is apparent, therefore, that the cottontail is either an incidental host to this species or it occurs sporadically in cottontails. It occurs normally in tame rabbits.

E. MEDIA FORM HONESSI. Oocysts of this form found in two cottontails were not infective to tame rabbits, and it therefore appears to be physiologically as well as morphologically differentiated from *media* as it occurs in tame rabbits.

3. THE SPECIES OF THE JACK RABBIT INTO TAME RABBITS AND COTTONTAILS

Several attempts were made to grow each of the species found in Iowa jack rabbits in tame rabbits and cottontails. All trials resulted in failure, even when young rabbits free from coccidia were used. This experimental work indicates that tame rabbits are more closely related to

cottontails than to jack rabbits. It also leads one to suspect the non-occurrence of the *Eimeria* species naturally found in tame rabbits in other species of jack rabbits. However, no jack rabbits were available for cross-infection trials, and no experimental assertion can be made in this connection. Further experiments on the subject are needed to establish this point.

SUMMARY AND CONCLUSIONS

1. This paper reports studies made by the author upon oocysts of *Eimeria* from the domestic rabbit, cottontail, and jack rabbit in Iowa, including experimental cross-infections with the different species.

2. Descriptions with comments, as well as figures of the oocysts, are presented for all species of *Eimeria* known from rabbits and hares.

3. *Eimeria americana* and *Eimeria minima* are being described as new species; *E. media* form *honessi*, *E. magna* form *townsendii* and *E. irresidua* form *campanius* are described as new forms.

Eimeria robertsoni is being raised to specific rank.

4. Of the cottontail species, *E. maior* and *E. environ* are here being reported for the first time from the Mearns cottontail and from Iowa, and *E. sylvilagi* is mentioned and found for the first time in the United States.

5. *E. robertsoni*, *E. sculpta*, and *E. septentrionalis* are also here being reported for the first time from jack rabbits in Iowa and from the United States.

6. Graphs with plotted dimensions of the species of *Eimeria* from cottontails and jack rabbits are annexed.

7. Experimental cross-infections were undertaken with the use of young rabbits free from coccidia as standard test-animals. Oocysts of all species from the jack rabbit were fed to young tame rabbits and cottontails, but results have shown that they are unable to grow in either host. The species of the tame rabbit were passed to cottontails, and vice versa. Although all species of the first host (excepting *E. stiedae* and *E. exigua*, not tried) were able to grow in cottontails, only *E. neoleporis* and *E. media* from the latter host could be grown in tame rabbits.

8. *E. magna* and *E. irresidua* from the jack rabbit were unable to grow either in tame rabbits or cottontails, and *E. media* form *honessi* from the latter host could not grow in tame rabbits, showing thus the presence of physiological species in the genus *Eimeria*, which are being treated in this paper under the category of forms.

9. These experiments prove once more that, based on the species of *Eimeria*, tame rabbits are more closely related phylogenetically to cottontails than to jack rabbits.

10. Experimental cross-infections have proved that in most species there are no biometrical or physiological changes in the parasite. In the case of *E. magna* in cottontails, however, there was not only an appreciable change in the length of the prepatent period, but also in the size of the oocysts. This leads to the assertion that in exceptional cases, size of oocysts alone may not be a good character in species differentiation.

HOST-CATALOGUE OF SPECIES OF THE GENUS *EIMERIA*
OCCURRING IN RABBITS AND HARES

- Lepus* (*Poecilolagus*) *americanus* Erxleben.
 E. perforans (Leuckart, 1879)
 E. stiedae (Lindemann, 1865)
 Reported by Boughton (1932)
- Lepus* (*Lepus*) *articus groenlandicus* Rhoads
 E. perforans var. *groenlandica* Madsen, 1938
 E. robertsoni Madsen, 1938
 E. septentrionalis Yakimoff et al., 1936
 E. sculpta Madsen, 1938
 Reported by Madsen, 1938
- Lepus* (*Macrotolagus*) *californicus* Gray
 E. irresidua Kessel & Jankiewicz, 1931
 E. magna Pérard, 1925
 E. media Kessel, 1929
 E. perforans (Leuckart, 1879)
 E. stiedae (Lindemann, 1865)
 Reported by Henry (1932)
- Lepus* (*Poecilolagus*) *townsendii campanius* Hollister
 E. americana n.sp.
 E. irresidua form *campanius* n. form
 E. magna form *townsendii* n. form
 E. septentrionalis Yakimoff et al., 1936
 E. sculpta Madsen, 1938
 Reported in the present paper.
- Lepus* (*Lepus*) *europaeus* Pallas
 E. leporis Nieschulz, 1923
 E. magna Pérard, 1925
 E. septentrionalis Yakimoff et al., 1936
 E. stiedae (Lindemann, 1865)
 Reported by several authors.
- Lepus* (*Lepus*) *timidus* Linnaeus
 The same species reported for *L. (L.) europaeus*.
 Reported by several authors.
- Oryctolagus cuniculus* (Linnaeus)
 E. exigua Yakimoff, 1934
 E. irresidua Kessel & Jankiewicz, 1931
 E. magna Pérard, 1925
 E. media Kessel, 1929
 E. perforans (Leuckart, 1879)
 E. stiedae (Lindemann, 1865)
 Reported by several authors.
- Sylvilagus* (*Sylvilagus*) *audubonii valicola* Nelson.
 E. stiedae (Lindemann, 1865)
 Reported by Jankiewicz (1941)

Sylvilagus (Sylvilagus) brasiliensis minensis (Oldfield)*E. paulistana* Fonseca, 1932*E. perforans* (Leuckart, 1879)*E. pintoensis* Fonseca, 1932*E. sylvilagi* Carini, 1940

Reported by Fonseca (1932-33) and Carini (1940).

Sylvilagus (Sylvilagus) floridanus mearnsii (Allen)*E. environ* Honess, 1939*E. maior* Honess, 1939*E. media* Kessel, 1929*E. media* form *honessi* n. form*E. minima* n. sp.*E. neoleporis* Carvalho, 1942*E. sylvilagi* Carini, 1940

Reported in the present paper.

Sylvilagus (Sylvilagus) nuttallii grangeri (Allen)*E. environ* Honess, 1939*E. maior* Honess, 1939*E. media* form *honessi* n. form*E. stiedae* (Lindemann, 1865)

Reported by Honess (1939).

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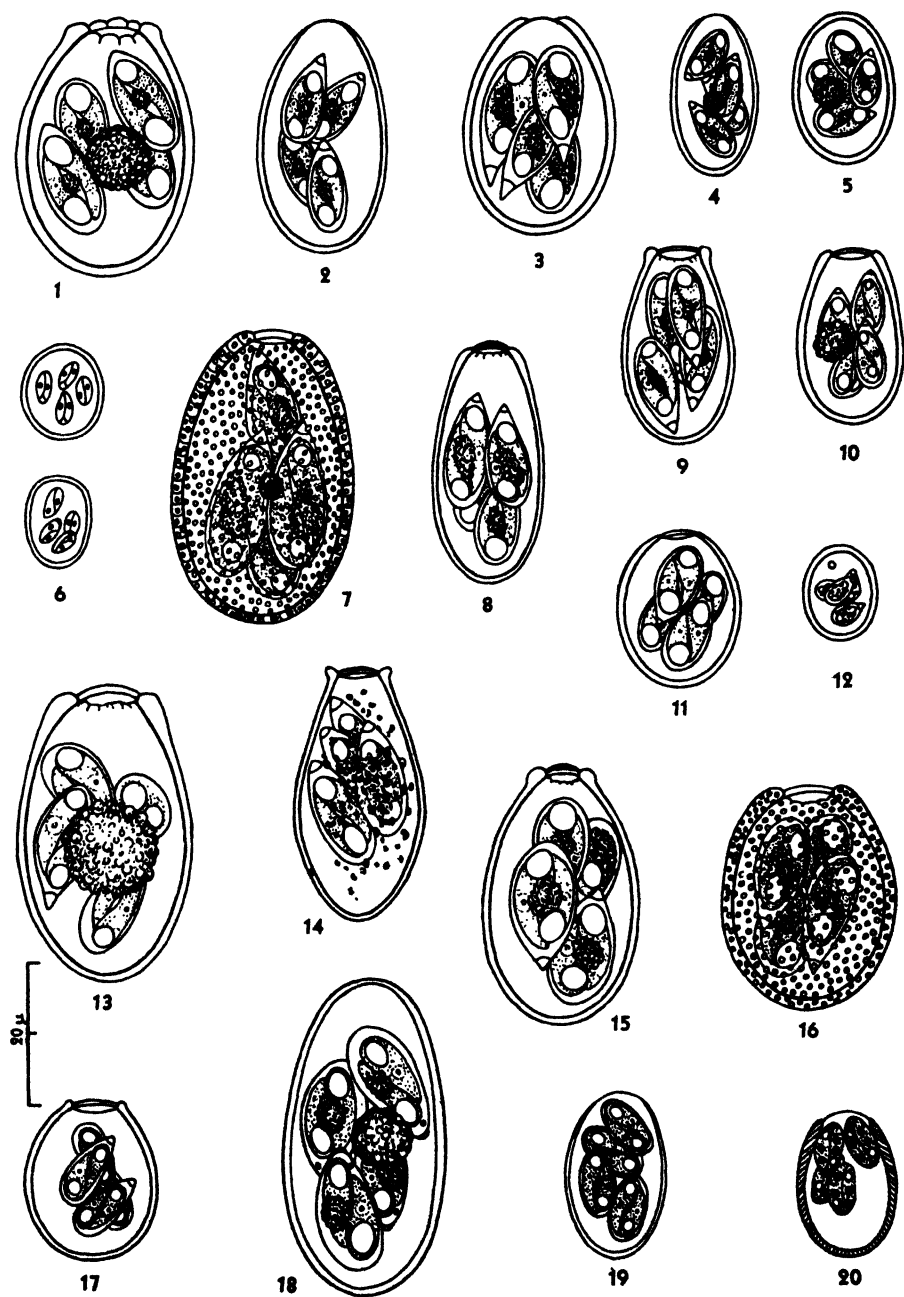
EXPLANATION OF PLATES

All figures were drawn under oil immersion with the aid of a camera lucida. The projected scale has the value (in micra) indicated in each plate.

PLATE I

- Fig. 1. *E. magna* Pérard, from the tame rabbit.
2. *E. stiedae* (Lindemann), from the tame rabbit.
3. *E. irresidua* Kessel & Jankiewicz, from the tame rabbit.
4. *E. media* Kessel, from the tame rabbit.
5. *E. perforans* (Leuckart), from the tame rabbit.
6. *E. exigua* Yakimoff, from the tame rabbit.
7. *E. maior* Honess, from the Mearns cottontail.
8. *E. neoleporis* Carvalho, from the Mearns cottontail.
9. *E. sylvilagi* Carini, from the Mearns cottontail.
10. *E. media* form *honessi* n. form, from the Mearns cottontail.
11. *E. environ* Honess, from the Mearns cottontail.
12. *E. minima* n.sp., from the Mearns cottontail.
13. *E. robertsoni* Madsen, from the jack rabbit.
14. *E. americana* n.sp., from the jack rabbit.
15. *E. irresidua* form *campanius* n. form, from the jack rabbit.
16. *E. sculpta* Madsen, from the jack rabbit.
17. *E. septentrionalis* Yakimoff *et al.*, from the jack rabbit.
18. *E. leporis* Nieschulz (redrawn after the original*).
19. *E. pintoensis* Fonseca, (redrawn after the original*).
20. *E. paulistana* Fonseca, (redrawn after the original*).

* Not according to scale.



FLORA OF ALASKA AND ADJACENT PARTS OF CANADA
AN ILLUSTRATED DESCRIPTIVE TEXT OF ALL VASCULAR PLANTS KNOWN
TO OCCUR WITHIN THE REGION COVERED

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PART 1

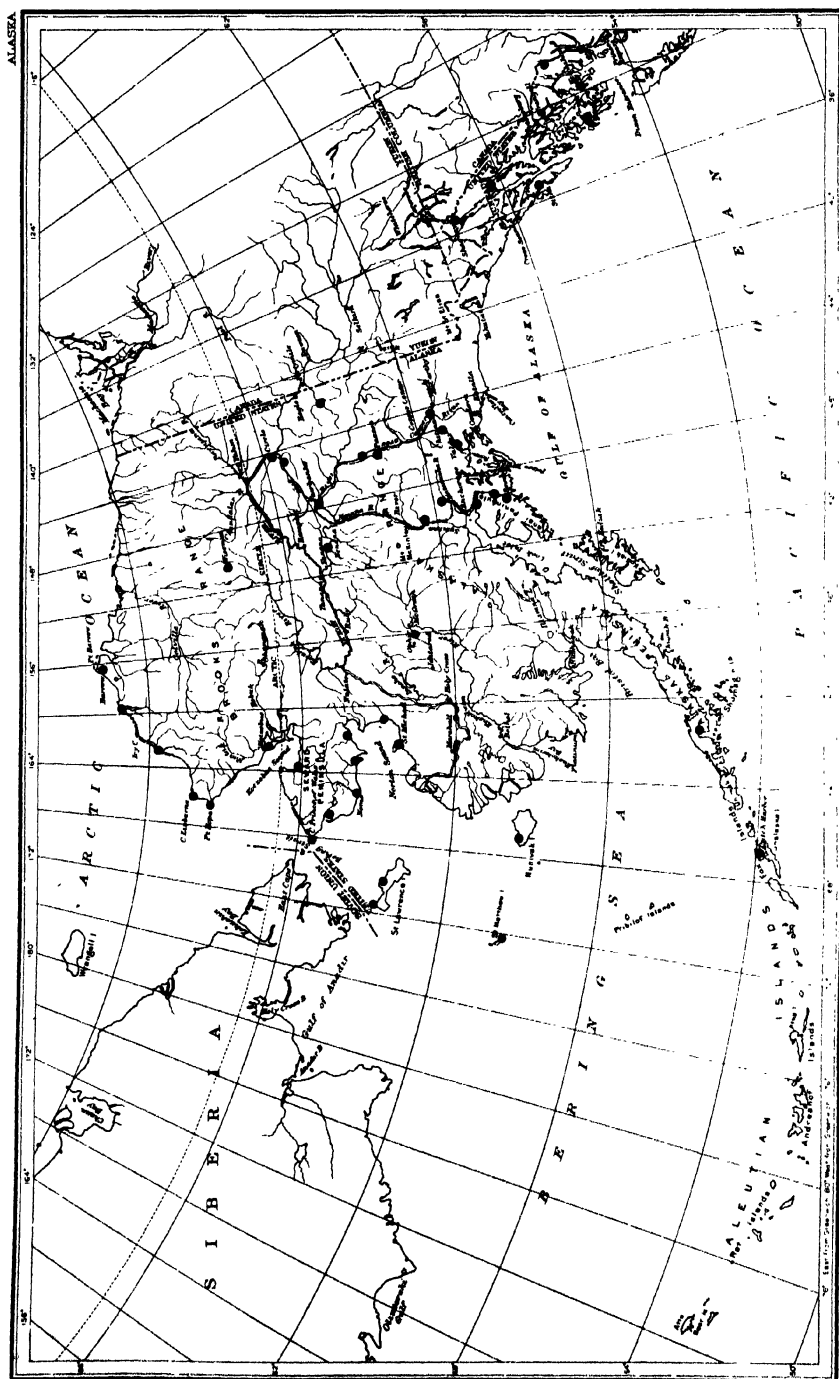
PTERIDOPHYTA AND GYMNOSPERMAE (FERNS TO CONIFERS)

INTRODUCTION

Few people realize the importance or the size and extent of Alaska. The region was originally considered most valuable for its furs, but fisheries and mining have long surpassed furs in value. Approximately 60 per cent of all the salmon canned in the whole world are caught in Alaskan waters, in addition to several million dollars worth of other fish and fish products. About \$25,000,000 in gold was produced in 1941 beside lesser values of many other mineral products. The value of Alaska in the defense of the United States is now beginning to be realized.

The actual area of Alaska is nearly 587,000 square miles which is more than twice that of Texas and ten and a half times that of Iowa. It extends from 130 degrees west longitude to 173 degrees east longitude or about the same as from Eastport, Maine to the Pacific coast of Oregon. Due to the actual length of degrees decreasing from the Equator to the Poles the actual distance in miles is about that from Savannah, Georgia, to Los Angeles, California. Point Barrow is about the same latitude as North Cape, Norway, and is about 20 degrees north of the middle Aleutian Islands. This distance is about the same as that from the Canadian border north of western Minnesota to the mouth of the Brazos River south of Houston, Texas. It may surprise many to learn that the middle Aleutian Islands extend about 3 degrees farther south than southeastern Alaska, and cut across the great circle route from Seattle or Vancouver to Yokohama.

Naturally in such an extended area climate varies. The Pacific Coast districts are characterized by mild winters and cool summers. Zero temperatures are rare and in some places unknown, while the average summer temperatures are between 50 and 60° F. Precipitation is heavy, and there is much cloudy weather. The eastern part of this region, from Cook Inlet eastward, is largely covered by a heavy forest growth, some trees reaching very large size. Along the Alaska Peninsula and the Aleutian Islands, summer conditions are unfavorable to tree growth, probably due to the excessively cool summers.



Stations where intensive collections were made by the author.

Following up the Bering Sea Coast we have a tundra-like formation merging into the true tundra farther north. These formations are treeless, covered in summer with a growth of low shrubs, grasses and sedges with intermixture of other herbaceous plants. In the true tundra the soil is permanently frozen, thawing a few feet at the surface each summer. The woody growth on these so-called barren lands consists mostly of dwarf birches, willows, and members of the Heather Family.

The interior districts are characterized by short, warm summers and long, cold winters. Summer temperatures are higher than in the coast districts, and the precipitation is comparatively light with long hours of sunshine. January temperatures in most places average below zero Fahrenheit. This region is largely covered by forest of moderate growth. Some limited districts, such as the head of Lynn Canal and Cook Inlet with the Matanuska Valley have climatic conditions intermediate between those of the coast and the interior.

From what has been said it can be perceived that there are three main types of vegetation in Alaska: 1. The heavily forested central and eastern Pacific Coast districts dominated by Sitka spruce and western hemlock. 2. The more lightly forested districts of the interior characterized by birches and white spruce. 3. The tundra and tundra-like districts of the Alaska peninsula, Aleutian Islands, Bering Sea littoral and Arctic slope. This latter type is also found in the other regions above timberline on the mountains, the alpine meadows occurring at successively lower altitudes until they meet the tundra. In types 1 and 2 are found muskegs or peat bogs. These are areas of a few square meters up, characterized by a surface covering of sphagnum moss underlaid with moss and other vegetation in various stages of decay, merging gradually into black muck, and the whole saturated with water. This mass of water-logged material may vary from less than 1 meter to many meters in depth. The surface usually is dotted with small ponds, and growing in the moss are very much stunted trees and characteristic shrubs and other plants, the whole having somewhat the appearance of tundra.

On February 1, 1914, the author started for Alaska. This was destined to be his home until October 1, 1941. The years 1914-16 were passed in Sitka as Horticulturist for the United States Agricultural Experiment Station. In 1917 he removed to Juneau and engaged in commercial floriculture under the title of Juneau Florists. In 1937 this business was sold with the idea of devoting more time to a study of the plant life of Alaska.

Having always been interested in plant life the author proceeded to collect the flora of the country as circumstances permitted. In November, 1924, the entire collection of more than 3,300 numbers was destroyed by fire. Some of these numbers were represented by specimens in the U. S. National Herbarium and elsewhere, but many were not. In 1925 a new collection was started which now numbers nearly 8,000; this, together with exchanges and specimens sent in by friends, brings the total of Alaska specimens in this herbarium up to about 10,000.

This collection is now permanently deposited at the Iowa State College of Agriculture and Mechanic Arts at Ames.

Besides places where residence was maintained, collections have been made at the following places: Ketchikan, Craig, Hyder, Skagway, Chitina, Valdez and along Richardson Highway to Fairbanks, Fairbanks and along Steese Highway to Circle, Franklin in the Fortymile district, Seward and other places on Kenai Peninsula, Matanuska Valley, Talkeetna, Healy, Manly Hot Springs, Wiseman, Takotna, Unalaska, St. Paul Island, Nunivak Island, St. Matthew Island, St. Lawrence Island, St. Michael, Stebbins, Unalakleet, Elim, Golovin, Nome, Teller, Cape Prince of Wales, Deering, Kotzebue, Kivelina, Point Hope, Cape Lisburne, Point Lay, Wainwright, and Barrow. Also fewer numbers were collected at other places and, by exchange and gift, specimens were obtained from places not visited by the author, especially from Mt. McKinley National Park and the Aleutian Islands.

Most of the literature dealing with the plants of Alaska is widely scattered in many publications, and at that is useful mostly to the professional botanist and not to the amateur. For this reason the author for some years has cherished the idea of writing a descriptive manual that, though scientifically accurate, would be so written that it would be of maximum usefulness to all persons interested in the flora, even though their botanical training was rather limited. The author has found many such persons, both among the permanent residents and among the tourists, who visit the territory every summer during normal times.

The author is not the only person working on the flora of Alaska. Dr. Eric Hultén of the Botanical Museum, Lund, Sweden, who is probably the world's foremost authority on arctic and boreal plants in general, in 1930 issued a "Flora of Kamtschatka" and in 1937 a "Flora of the Aleutian Islands." These are very good in listing the known species, presenting data on collections and giving useful comments, but contain no keys or descriptions, except of new species or varieties. Dr. Hultén is now at work on the "Flora of Alaska and Yukon," and the first part listing ninety-one species has been issued. This is on the same plan as his other publications listed above but does contain keys to the species but not to the families or genera. The author has consulted these works of Dr. Hultén and places much reliance on them. In addition, leading American manuals have been freely consulted, also papers on special groups in leading scientific publications, and lists of plants made by various collectors in Alaska.

Although the author's primary interest is in Alaska, species known to occur in adjacent parts of Canada are included. This additional area is Yukon Territory and the extreme northwestern part of British Columbia. These regions belong to the same floral provinces, and nearly all form a part of the Yukon River drainage system. The entire area covered is more than 800,000 square miles.

This manual aims to include all species of vascular plants known to

occur within the geographical limits covered. In such a vast region, so sparsely settled, there remain large districts in which there has been no botanical collecting. Even in places where considerable collections have been made, other species may be found. The author collected in the vicinity of Juneau for many years, and every season when considerable scouting around was possible, species not before noted in the region were found. It is evident, therefore, that many more species will be found as further collections and studies are made. However, enough is known to give a good general idea of the flora and to include all the more common and widely distributed species.

The arrangement of the families in this manual is that usually followed in American descriptive manuals and the herbaria of the country, although it does not always reflect the true relationships.

The nomenclature followed is that known as the International Rules. The original rules as adopted in 1905 have been greatly modified and modernized by International Botanical Congresses since that time and are now generally used by American botanists. The synonyms as given are not complete, but include only those used in publications relating to Alaska or in American or Canadian descriptive manuals. Further, all names given are not necessarily synonymous, but the species may have been reported under that name due to error in determination, hence a synonym only in that sense.

Unless otherwise noted all illustrations were made by the author and are based on material collected in Alaska. The aim has been to make them as true to nature as possible and to bring out the differences between the species more clearly than short descriptions make possible.

In giving the range of a species the abbreviations of the states and Canadian provinces are those in general use. Circumboreal does not mean that the distribution is necessarily continuous around the northern regions, for many species have breaks in this continuity of occurrence. They do, however, occur in some part of North America, Europe, and Asia.

Measurements are given in the metric system. The following are the approximate equivalents in inches; 25 millimeters (mm.) equals 1 inch, 1 decimeter (dm.) equals 4 inches, 1 meter (m.) equals 39.37 inches.

As the completion of this manual will require several years, it has been thought best to publish it in parts as ready, so as to make such parts available to interested parties at once. This is all the more desirable as all published American manuals combined do not even mention some of the species occurring in Alaska, especially the western part of the territory.

Index and other accessory material will accompany the completed manual.

ACKNOWLEDGEMENTS

This work is being carried on at the Iowa State College of Agriculture and Mechanic Arts where the author moved his botanical col-

lections in 1941 in order to have better facilities for this work. The facilities of the College were placed at his disposal, and the members of the Botanical staff have been helpful, especially the head of the Botany Department, Dr. I. E. Melhus, Dr. Geo. J. Goodman, Curator of the Herbarium, and Dr. J. C. Gilman, Editor of Iowa State College Journal of Science.

PHYLUM PTERIDOPHYTA

Plants containing woody and vascular tissues in the stem and producing spores which give rise to small, inconspicuous growths known as prothallia (gametophytes), on which the archegonia (female organs) and antheridia (male organs) are borne. The fertilization of an archegonium by a spermatozoid from an antheridium results in the large conspicuous plant we call a fern, a horsetail or a club-moss.

Known living species number about 7,000, three-fourths of them tropical. They appeared early in geological history, and were the predominant type of vegetation during the Carboniferous times, fossil forms often being found in connection with coal beds. This was probably about 250 million years ago.

- 1A. Spores produced in sporangia, which are borne on the back of the leaf, in spikes or panicles Order 1. *Filicales*
 - 1B. Vernation erect or inclined, sporangia in spikes or panicles. Family 1. *Ophioglossaceae*
 - 2B. Vernation coiled, sporangia reticulated Family 2. *Polypodiaceae*
- 2A. Spores produced in sporangia, which are clustered underneath scales in a terminal conelike spike Order 2. *Equisetales*
 - One family Family *Equisetaceae*
- 3A. Spores produced in sporangia, which occur in the axils of scalelike or tubular leaves. Order 3. *Lycopodiales*
 - 1B. Spores all of one size. Family 1. *Lycopodiaceae*
 - 2B. Spores of two sizes:
 - 1C. Leaves scalelike. Family 2. *Selaginellaceae*
 - 2C. Leaves tubular. Family 3. *Isoetaceae*

OPHIOGLOSSACEAE (Adder's-tongue Family)

More-or-less succulent plants with fleshy rhizomes and stems bearing a leaf and one or more stalked spore-bearing spikes or panicles (sporophylls). Leaves simple or usually compound, not coiled in vernation, sporangia bivalvular, formed from the interior tissues of the sporophylls; prothallia subterranean, without chlorophyll.

Veins reticulate; sporangia in a spike. 1. *Ophioglossum*

Veins free; leaves pinnatifid to tripinnate;

sporangia in panicles. 2. *Botrychium*

1. OPHIOGLOSSUM (Tourn.) L.

Small herbaceous perennials with short, usually erect, fleshy subterranean rhizomes. Leaves erect, glabrous, fleshy, arising at the side of the apical bud; sterile blade simple, sessile or short-stalked, with reticulate venation; sporophyll a simple, slender, long-stalked spike; the large globose sporangia marginal in two ranks, transversely dehiscent. (Greek, tongue of a snake, alluding to the sporophyll.)

O. vulgatum L.

Adder's-tongue

Fronds usually solitary, 1–4 dm. tall; sterile blade usually sessile, lanceolate to spatulate or ovate, $2\frac{1}{2}$ –12 cm. long \times 1–5 cm. broad; spike 2–4 cm. long \times $1\frac{1}{2}$ – $3\frac{1}{2}$ mm. wide, apiculate; sporangia 10–30 pairs. Our plant differs from the type in its large, thin, ovate, very distinctly veined sterile blade. It was described by E. G. Britton as *O. alaskanum* and may be regarded as a variety of the circumboreal *O. vulgatum*. It has been twice collected at Unalaska.

2. BOTRYCHIUM Sw.

Rootstock short, erect, with fleshy clustered roots, the bud for the succeeding season's frond embedded in the base of the stem; the blade pinnately or ternately compound; veins free, forking; the sporophylls pinnate to tripinnate with sessile, distinct sporangia on either side of its branches, forming large panicles in some species. (Name in allusion to the grapelike arrangement of the sporangia.)

1A. Sterile blade once to twice pinnate.

- | | |
|---|--------------------------|
| 1B. Segments reniform or fan-shaped. | 1. <i>B. lunaria</i> |
| 2B. Segments rounded. | 2. <i>B. boreale</i> |
| 3B. Segments acute. | 3. <i>B. lanceolatum</i> |

2A. Sterile blade thrice pinnate.

- | | |
|--|--------------------------|
| 1B. Sterile blade long-petioled, arising from the base of the plant.
..... | 4. <i>B. silaifolium</i> |
| 2B. Sterile blade sessile or nearly so, affixed to middle of the plant.
..... | 5. <i>B. virginianum</i> |

1. *B. lunaria* (L.) Sw.

Moonwort

Fleshy, 4–30 cm. tall; sterile leaf-blade nearly sessile, borne about the middle of the plant, simply pinnatifid, the segments lunate or fan-shaped, entire or crenulate or even incised, often imbricate; sporophyll bent down in vernation, at maturity erect and surpassing the sterile blade; panicle 1–3 times pinnate. A circumboreal species occurring in Alaska from about the Arctic circle southward. (Fig. 1.)

2. *B. boreale* (Sw.) Milde.

Northern Grape-fern

Fleshy, 4–25 cm. tall; sterile leaf nearly sessile, borne above the middle of the plant, triangular in outline, obtuse, pinnatifid, with the lower divisions crenately incised, all divisions crenate and often imbricate.

cate; sporophylls much as in *B. lunaria*. A form found at Unalaska has been described as var. *obtusilobium* Rupr. Circumboreal, in Alaska from Wiseman southward. (Fig. 2.)

3. *B. lanceolatum* (S. G. Gmel.) Angstr. Lance-leaved Grape-fern

Fronds 5-20 cm. tall; common stalk long; sterile leaf sessile, triangular, acute, 1-6 cm. long \times 1-8 cm. wide, once or twice pinnately divided, the primary divisions ovate-lanceolate; sporophylls sessile or short-stalked, forming a diffuse panicle, the larger divisions ascending and often subequal. Arctic-alpine situations, Unalaska eastward. Circumboreal. (Fig. 3.)

4. *B. silaifolium* Presl.

Leathery Grape-fern

B. multifidum (Gmel.) Rupr. var. *robustum* (Rupr.) C. Chr.

Fronds 1-6 dm. tall, fleshy, coriaceous in drying; sterile leaf long-stalked, broadly triangular or pentagonal in outline, 10-30 cm. broad and about as long, subternately compound, the primary divisions 1-3-pinnately divided, the ultimate segments ovate or rhomboid, crenulate, obtuse; sporophyll long-stalked with diffuse panicle. Known from the Aleutian Islands and southeastern Alaska thence to Quebec.—Pa.—Wis.—N. Calif. (Fig. 4.)

5. *B. virginianum* (L.) Sw.

Virginia Grape-fern

Fronds 1-7 dm. tall; stalk slender; sterile blade sessile or nearly so, spreading, thin and membranous, deltoid, 4-21 cm. long \times 5-36 cm. wide, the ultimate divisions variously toothed or lobed; sporophyll long-stalked, 2-3-pinnate. Isolated and rare in southwestern Alaska. Occurs B.C.—Lab.—Fla. Also in Eurasia, Brazil, and Mexico.

POLYPODIACEAE (Fern Family)

Leafy plants with the rootstocks horizontal, often elongated, or shorter and oblique or erect, often stout; the leaves (fronds) coiled in the bud. Sterile fronds leaflike; fertile fronds (sporophylls) leaflike or more or less modified, bearing the sporangia on their lower surface or at their margins, usually in clusters (sori); sori naked or usually covered, especially when young, by a membrane (indusium); sporangia stalked, furnished with an incomplete ring of thickened cells (annulus), opening transversely; prothallia green, above ground.

1A. Sterile and fertile fronds different, pinnae of fertile fronds contracted.

1B. Fertile fronds simply pinnate.

1C. Sterile fronds simply pinnate. 1. *Blechnum*

2C. Sterile fronds with pinnatifid pinnae. 2. *Struthiopteris*

2B. Sterile and fertile fronds bipinnate. 3. *Cryptogramma*

2A. Sterile and fertile fronds similar.

1B. Sori marginal, the indusia formed wholly or in part by the revolute leaf margins.

- 1C. Sori distinct, on underside of reflexed leaf-lobes. 4. *Adiantum*
- 2C. Sori continuous or confluent. 5. *Pteridium*
- 2B. Sori dorsal on the veins.
 - 1C. Sori roundish.
 - 1D. Sori naked. 6. *Polypodium*
 - 2D. Sori with wholly or partly inferior indusia.
 - 1E. Indusia wholly inferior, the divisions stellate or hairlike. 7. *Woodsia*
 - 2E. Indusia hood-shaped, attached at side, early deciduous. 8. *Cystopteris*
 - 3D. Indusia superior.
 - 1E. Indusia peltate, centrally attached. 9. *Polystichum*
 - 2E. Indusia orbicular-reniform, attached at the sinus. 10. *Dryopteris*
 - 2C. Sori oblong.
 - 1D. Sori straight or slightly curved, fronds evergreen. 11. *Asplenium*
 - 2D. Sori usually curved, fronds herbaceous. 12. *Athyrium*

1. BLECHNUM (L.) With.

Our species is a woodland fern with woody rootstock and fronds of two kinds, both with pinnate or pinnatifid blades. Sori in a continuous band next to the midrib, covered by a continuous membranous indusium arising under the margin of the pinna; indusium often lacerate, often reflexed at maturity. (Greek for some fern.)

B. spicant (L.) J. E. Smith.

Deer-fern

Lomaria spicant (L.) Desv.

Osmunda spicant L.

Struthiopteris spicant (L.) Weis.

Sterile fronds numerous, in a circular crown, evergreen; 2–10 dm. long; stipe rather short, brownish; blades linear-lanceolate, attenuate to both ends, cut to the rachis into linear, falcate segments, those near the base mere auricles, the segments entire or finely crenulate toward the apex; fertile fronds few, central, erect, 4–15 dm. long, with long reddish-brown stipes; blades pinnate, the pinnae narrowly linear.

Known from Atka and Kodiak Islands and common in the coast region from Cook Inlet eastward and extending to California. Also in Eurasia. (Fig. 5.)

2. STRUTHIOPTERIS Scop.

Coarse ferns with the fertile fronds rolled into necklace-like or berry-like segments and unlike the foliaceous sterile ones; sori round,

borne on the back of the veins; indusium delicate, fixed at the inferior side of the sorus. (Name from *struthio*, ostrich; and *pteris*, fern.)

S. filicastrum All.

Ostrich-fern

Matteucia struthiopteris (L.) Todor.

Onoclea struthiopteris (L.) Hoffm.

Pteretis nodulosa (Michx.) Nieuland.

Rootstock stout, bearing a circle of sterile fronds with fertile ones in the center; sterile fronds up to 2 meters tall, narrowed at the base, pinnate, the pinnae once pinnatifid, 5–18 cm. long; fertile fronds shorter, with rigid upcurved, necklace-shaped pinnae; veins pinnate, free, simple; texture firm.

Woods; central and southern Alaska, especially abundant along the Alaska railroad from Talkeetna to north of Curry. Distribution circumboreal. (Fig. 6.)

3. CRYTOGRAMMA R. Br.

Small ferns of rocky situations with dimorphous, tufted, 2–3-pinnate fronds. Sterile fronds foliaceous, with numerous, crowded, rather small, obtuse segments; sori in a continuous line at the free ends of the forked veins, confluent; indusia formed of the revolute, modified margins of the segments, which later open out. (Greek, in allusion to the hidden sori.)

Rootstock stout, short. 1. *C. acrostichoides*

Rootstock slender, creeping. 2. *C. stelleri*

1. *C. acrostichoides* R. Br.

Parsley-fern

Rhizome in massive tufts, chaffy; fronds numerous, the fertile 1–3 dm. long, erect, long-stalked, overtopping the short-stalked sterile ones; sterile blades ovate to ovate-lanceolate, the ultimate segments suboval, obtuse, serrulate; fertile segments elliptical or linear, 6–12 mm. long, about 2 mm. wide. Considered by some botanists as only a variety or subspecies of the Eurasian *C. crispa* (L.) R. Br.

Pacific coast and Bering Sea regions of Alaska—Baffin Bay—Colo.—Calif. (Fig. 7.)

The var. *sitchense* (Rupr.) C. Chr. is characterized by the broadly deltoid tripinnate sterile fronds with small, more deeply toothed segments. Same range.

2. *C. stelleri* (S. G. Gmel.) Prantl.

Slender Cliff-brake

Fronds scattered, arising singly from slender creeping rhizomes; pinnae few, the lower ones usually pinnatifid; segments of the sterile blades ovate or obovate, crenately lobed; those of the fertile ones linear to lanceolate.

East central Alaska—Lab.—Va.—Colo.—Wash. (Fig. 8.)

4. ADIANTUM (Tourn.) L.

Graceful delicate ferns of moist rocky woods and ravines with compound fronds having segments in the form of small leaflets and with

dark-colored shining stipes. Sori marginal under the modified, sharply reflexed margins of the leaflets. (Greek, unwetted, in allusion to the leaflets repelling raindrops.)

A. pedatum L.

Maiden-hair Fern

Rhizome thickish, chaffy with shining, dark, chestnut-brown scales; fronds 2–8 dm. long, forking into 4–8-pinnate divisions, the longer ones 1–3 dm. long; segments very short-stalked, the lower margin formed by the midrib, the upper cut and toothed.

Coastal districts of Alaska—N. S.—Ga.—Ark.—Calif.—Asia. The form on the Pacific coast from Japan to Alaska to Calif. and on the Atlantic coast from Newf. to Mass. has pinnules with longer stalks and the upper margin more deeply cleft. It has been described as the var. *aleuticum* Rupr. (Fig. 9.)

5. PTERIDIUM Scop.

Coarse ferns of open or partly shaded situations with woody, branched, wide-creeping rhizomes. Sporangia borne in a continuous line under the margin of the frond, occupying a veinlike receptacle connecting the ends of the veins; indusium double, the outer one formed by the reflexed margin of the frond, the inner delicate and minute. (Diminutive of pteris, Greek name of ferns.)

P. aquilinum (L.) Kuhn var. *lanuginosum* Bong.

Western Bracken

P. aquilinum (L.) Kuhn var. *pubescens* Underw.

Stipe erect, stout, 15–100 cm. long; blades triangular or deltoid-ovate, as long or longer than the stipe, subternately tripinnate, the lower divisions being bipinnate; segments variable, mostly oblong and entire, pubescent or strongly tomentose beneath, slightly hairy or glabrous above.

Southeastern Alaska—Mont.—Mex. Entire species quite cosmopolitan in distribution. (Fig. 10.)

6. POLYPODIUM (Tourn.) L.

Ferns of various habit, our species with creeping rootstock growing in moss. Fronds pinnately compound, usually articulated to the rhizome; sori round or elliptical, borne on the backs of the fronds, without indusia, veins free. (Greek, many and foot alluding to the knoblike prominences of the rhizome).

P. vulgare L. var. *occidentalis* Hook.

Licorice-fern

P. glycyrrhiza D. C. Eat.

P. falcatum Kell.

Rhizome hard, 3–5 mm. thick, covered with rusty-brown scales; fronds 1–6 dm. long, the stipe usually shorter than the blade, firm, naked; blades lanceolate, abruptly attenuate or caudate, pinnatisect; segments alternate, tapering from the middle or the base, serrulate; sori about midway between midrib and edge of the segments.

Common in the coastal districts and rare in the Yukon Valley, extending to California. Entire species circumboreal. (Fig. 11.)

7. WOODSIA R. Br.

Small ferns of rocky situations with densely tufted, pinnately compound fronds and round sori borne on the back of the free veins. Indusia placed under the sporangia, thin and often evanescent, roundish or stellate, small and open, or bursting at the top into irregular segments. (Joseph Woods (1776–1864) was an English architect and botanist.)

1A. Stipe articulated near the base.

1B. Fronds glabrous. 1. *W. glabella*

2B. Fronds with hairs or scales on the lower surface.

1C. Primary segments about as broad as long. 2. *W. alpina*

2C. Primary segments longer than broad. ... 3. *W. ilvensis*

2A. Stipe not articulated near the base. 4. *W. scopulina*

1. *W. glabella* R. Br.

Smooth Woodsia

Fronds tufted, pinnate, 3–16 cm. long; stipes smooth, usually straw-colored; pinnae deltoid to ovate, crenately lobed or parted, glabrous; indusia divided into narrow, hairlike, curving divisions.

Moist rocks, in most parts of our territory. Circumboreal. (Fig. 12.)

2. *W. alpina* (Bolton) S. F. Gray.

Alpine Woodsia

Rootstock short; fronds densely tufted, the blades narrowly lanceolate, 5–15 cm. long \times 15–25 mm. wide; pinnae cordate-ovate to triangular-ovate, pinnately 5–7-lobed, sparingly hairy; sori near the margins, the indusia cleft into numerous hairlike filaments.

Moist rocks, of scattered distribution through most of Alaska and Yukon Ter. Circumboreal. (Fig. 13.)

3. *W. ilvensis* (L.) R. Br.

Rusty Woodsia

Fronds tufted, lanceolate, 8–20 cm. long; pinnae pinnately lobed, sparingly hairy above, hairy and with rusty chaff beneath; sori borne near the margins of the segments, somewhat confluent when old; indusia cleft into filiform segments.

In most of Alaska south of the Arctic Circle. Circumboreal. (Fig. 14.)

4. *W. scopulina* D. C. Eat.

Rocky Mountain Woodsia

Fronds numerous, borne close together, 6–35 cm. long, blades lanceolate, finely glandular-puberulent; pinnae oblong-ovate, deeply pinnatifid into 10–16-toothed segments; indusia delicate, cleft into narrow, spreading, flaccid segments.

Crevices of rocks, Kenai Penin. and southeastern Alaska—Calif.—Utah—S. Dak. Isolated station in eastern America.

8. CYSTOPTERIS Bernh.

Ferns of rather thin texture, on slender stipes with 2–4-pinnate blades. Sori roundish, borne on the backs of the veins; indusia delicate,

hoodlike or flattish, attached at one side and partly underneath the sori, at first arched over them, later thrown back and withering, the sori then appearing naked. (Greek, bladder-fern.)

- Blades lanceolate, 2-3-pinnate.1. *C. fragilis*
 Blades deltoid, 3-4-pinnate.2. *C. montana*

1. *C. fragilis* (L.) Bernh.

Fragile-fern

Filix fragilis (L.) Gilib.

Fronds somewhat clustered or slightly scattered; stipe slender, about as long as the blade, brittle, stramineous or brownish below; blades extremely variable, nearly or fully bipinnate; pinnae deltoid to lanceolate or ovate-lanceolate, acute to acuminate, narrowly decurrent on the rachis, the lower ones slightly reduced; the segments toothed or incised; veinlets excurrent to the marginal teeth; indusia convex, rounded or usually pointed, toothed or lacerate at apex.

Common and very variable. The most widely distributed and cosmopolitan of all ferns. (Fig. 15.)

2. *C. montana* (Lam.) Bernh.

Mountain Cystopteris

Filix montana (Lam.) Underw.

Rhizomes slender, widely creeping; fronds scattered, stipe slender, blade often subternate, 3-4-pinnate, 5-15 cm. long and wide; lower pinnae much the largest; the pinnules pinnatifid to the winged rachis, the final segments oblong, deeply toothed or divided; indusia convex, acute, soon thrown back or withering.

Bering Str. to Yukon Ter. Circumboreal. (Fig. 16.)

9. POLYSTICHUM Roth.

Ferns of rather firm texture, with pinnate or pinnately decompound, tufted fronds from the crown of the rhizome, the divisions with sharply toothed or spinulose margins (except in *P. aleuticum*); sori round, indusia peltate, attached by the middle, persistent to caducous; veins free. (Greek, many rows.)

1A. Blades simply pinnate.

- 1B. Low-grown, tissues thin. 1. *P. aleuticum*
 2B. Taller, coriaceous, pinnae with spinulose teeth.
 1C. Fronds short-stalked, lower pinnae reduced. 2. *P. lonchites*
 2C. Fronds longer stalked, lower pinnae about as long as those
 above. 3. *P. munitum*

2A. Blades bipinnate.

- 1B. First upturned secondary segment longer than the others.....
 4. *P. andersoni*
 2B. First upturned secondary segment not conspicuously longer
 than the others 5. *P. braunii*

1. *P. aleuticum* C. Chr. Aleutian Shield-fern

Fronds about 15 cm. tall, blades thin, pinnae not spinose or aristate and with the general appearance of *Woodsia alpina*.

Known only from a single collection made on Atka island.

2. *P. lonchites* (L.) Roth. Holly-fern

Fronds rigidly ascending in a close crown, 1-6 dm. tall, bearing pinnae almost to the base, densely chaffy at base, lanceolate in outline, broadest near the middle; rachis more or less chaffy; pinnae numerous, close, densely spinulose-toothed, glabrous above, somewhat chaffy beneath; auricles on upper side, sori usually in two rows, indusia orbicular, nearly entire.

Woods, Pacific coast districts. Circumboreal. (Fig. 17.)

3. *P. munitum* (Kaulf.) Presl. Dagger-fern

Fronds growing in a crown, 3-15 dm. tall; stipes 5-60 cm. long, together with the rachis decidedly chaffy; blades lanceolate, narrowed toward the base; pinnae numerous, spreading, 2-14 cm. long, sharply and often doubly serrate, the serrations with spinescent, often incurved teeth; indusia papillose-dentate to long ciliate.

In woods, southeastern Alaska—Mont.—Calif. (Fig. 18.)

4. *P. andersonii* Hopkins. Anderson's Shield-fern

Similar in appearance to *P. braunii* but the rachis with proliferous buds, the first upturned pinnule conspicuously larger than the next, and the base of the pinnules decurrent and connecting, the blade scarcely bipinnate. Indusia ciliate-erose.

A rather rare woodland fern in southeastern Alaska and ranging to Mont. and Wash.

5. *P. braunii* (Spencer) Fée. Prickly Shield-fern

P. alaskense Maxon.

Fronds in a crown, 2-6 dm. tall; stipe and rachis chaffy with both broad and narrow bright-brown scales; blades lanceolate, gradually narrowed toward the base; pinnae numerous, lanceolate; segments ovate, oblique, spinulose-toothed, beset with long, soft hairs and scales; indusia orbicular, small, nearly entire.

In woods, Pacific coast districts of Alaska. Circumboreal. (Fig. 19.)

The form described as *P. alaskense* may be regarded as a variety. It has pinnules which are more cuneate at the base, more ellipsoid in form, and have a broader attachment at the base.

10. DRYOPTERIS Adans.

Aspidium Sw. in part.

Mainly woodland ferns of upright growth; rhizomes various, fronds borne singly or in a crown, the fertile and sterile usually alike, 1-3-pinnate or decompound; sori roundish, dorsal; indusia when present roundish-reniform, fixed at its sinus. (Greek, meaning oak-fern.)

- 1A. Blades long-stalked, triangular, fronds scattered.
 1B. Blades longer than broad, pinnate-pinnatifid. 1. *D. phegopteris*
 2B. Blades as broad as long, 2-3-pinnate.
 1C. Fronds glandless.2. *D. linnaeana*
 2C. Rachis and lower surface glandular.3. *D. robertiana*

2A. Blades clustered from short stout rhizomes.

- 1B. Blades 1-2-pinnate.
 1C. Blades small, thick.4. *D. fragrans*
 2C. Blades large, thin.5. *D. oreopteris*
 2B. Blades 2-3-pinnate.6. *D. austriaca*

1. *D. phegopteris* (L.) C. Chr. Beech-fern
Phegopteris phegopteris (L.) Underw.
Thelypteris phegopteris (L.) Slosson.

Rhizome slender, wide-creeping; fronds scattered, 10-55 cm. long, the stipe usually longer than the blade, more or less scaly; blades triangular, long-acuminate, sparingly hairy on both surfaces, especially on the veins; pinnae mostly closely adnate, horizontal, linear-lanceolate, pinnatifid; segments oblong, obtuse, entire or slightly crenate; sori submarginal, naked.

In woods, most parts of Alaska—Greenl.—Newf.—Va.—Ohio—Wash. (Fig. 20.)

2. *D. linnaeana* C. Chr. Oak-fern
D. dryopteris (L.) Christ.
Phegopteris dryopteris (L.) Fée.
Thelypteris dryopteris (L.) Slosson.

Rhizomes slender, wide-creeping; fronds scattered, erect, 1-6 dm. long; stipe slender, much longer than the blade, from a chaffy blackish base; blades deltoid, 8-25 cm. long and wide, subternate, the 3 primary divisions stalked, 1-2-pinnate, the larger pinnules pinnately lobed or divided; lobes oblong, entire to serrate-crenate; sori small, without indusia; leaf tissue thin, glabrous.

In woods, common in most parts of Alaska. Circumboreal. (Fig. 21.)

3. *D. robertiana* (Hoffm.) C. Chr. Scented Oak-fern
Thelypteris robertiana (Hoffm.) Slosson.

Very similar to *D. linnaeana* in appearance but the stipe and blade bearing minute stalked glands. The lateral main divisions of the blade are also somewhat smaller in proportion.

In woods, central Yukon River district. Circumboreal.

4. *D. fragrans* (L.) Schott. Fragrant Shield-fern
D. aquilonaris Maxon.
Aspidium fragrans (L.) Sw.

Rhizome chaffy with brown shining scales; fronds borne in a dense crown, 4-40 cm. long, aromatic; stipe short and with the rachis very

chaffy; blade lanceolate, narrowed toward the base, bipinnate; pinnae triangular-lanceolate, the segments oblong, obtuse, adnate-decurrent, dentate or nearly entire; nearly covered by the large sori; indusia very large, persistent, ragged, somewhat glandular.

On rocks, Bering Sea—Wiseman—Matanuska east. Circumboreal. (Fig. 22.)

5. *D. oreopteris* (Ehrh.) Maxon.

Mountain Wood-fern

Thelypteris oreopteris (Ehrh.) Slosson.

Aspidium oreopteris (Ehrh.) Sw.

Fronds in a crown, ascending, glandular, 4–11 dm. long; stipes short, stipe and rachis somewhat scaly; blades lanceolate, tapering below; pinnae pinnatifid, broadest at base, glabrous or nearly so above, sometimes short-hairy on the veins and midrib below; segments oblong, obtuse, subentire, the margins finely hyaline-papillose; sori rather small, submarginal; indusia round-reniform, toothed, deciduous.

On mountain slopes, Pacific coast districts of Alaska. Circumboreal. (Fig. 23.)

6. *D. austriaca* (Jacq.) Woyнар.

Spreading Wood-fern

D. dilatata (Hoffm.) A. Gray.

D. spinulosa (Muell.) Kuntze.

Aspidium spinulosum var. *dilatatum* Hook.

Rhizome chaffy; fronds in a crown, 3–12 dm. long, stipe stout, 15–50 cm. long, chaffy with brownish, often darker-centered scales; blades triangular to ovate, acuminate, nearly or fully tripinnate; pinnae unequally ovate or triangular; pinnules lanceolate to oblong, the larger ones not decurrent, pinnate or pinnately divided; the ultimate segments pinnatifid or toothed; indusia glabrous or sparsely glandular.

In woods, from Bering Sea east and south. Circumboreal. (Fig. 24.)

11. ASPLENIUM L.

Our species are small ferns of rocky ledges with simply pinnate leaves. Sori oblong or linear, oblique, borne on the veins; indusia straight or curved, attached by one edge, often nearly concealed by the sporangia at maturity. (Greek, alluding to the supposed medicinal properties.)

Rachis dark brown, shining.1. *A. trichomanes*

Rachis yellowish-green.2. *A. viride*

1. *A. trichomanes* L.

Maidenhair Spleenwort

Fronds tufted, 5–20 cm. long; stipes short; blades linear, somewhat narrowed toward base and apex; pinnae oval or oval-oblong, 3–8 mm. long, rigid, evergreen, sessile, the margins usually crenulate; indusia usually crenulate.

Southeastern Alaska, rare. Circumboreal—Africa—Australia—S. Am. (Fig. 25.)

2. *A. viride* Huds.

Green Spleenwort

Fronds tufted, 4-20 cm. long, laxly ascending; stipes 1-7 cm. long, reddish-brown at base only; blades linear-lanceolate, pinnae up to 25 pairs, roundish-ovate to rhombic, obtuse, cuneate at base, the margins deeply crenate; sori at maturity becoming confluent, concealing the delicate indusia.

Pacific and Bering Sea districts. Circumboreal. (Fig. 26.)

12. *ATHYRIUM* Roth.

Medium to large ferns of upright habit growing in moist situations. Fronds usually large, long-stipitate, erect-spreading, ours 2-3-pinnate; veins free; sori dorsal, oblique to the midrib, oblong, or often crossing the vein and becoming horseshoe-shaped or roundish; indusia following the shape of the sori, attached along its length at the side next to the vein, delicate, sometimes minute or hidden. (Greek, shield-less, which seems hardly applicable.)

Fronds bipinnate 1. *A. filix-femina*
Fronds tripinnate or nearly so. 2. *A. alpestre*

1. *A. filix-femina* (L.) Roth.

Lady-fern

A. filix-femina (L.) Roth. var. *sitchense* Rupr.

A. cyclosorum Rupr.

"*Asplenium cyclosorum* Rupr." (Henry's Flora of Southern B. C.)

Rhizomes erect or ascending, stout; fronds closely clustered, up to 2 m. long; stipes straw-colored, dark at base; blades lanceolate, attenuate toward both ends; pinnae linear to lanceolate, attenuate or acuminate, sessile; segments from crenate to incised or pinnatifid and dentate; sori oblong, linear or horseshoe-shaped; indusia subentire to toothed or ciliate. Our form is perhaps best classified as var. *cyclosorum* (Rupr.) C. Chr.

Central Alaska and Bering Sea—Wash.—Ida. The entire species is circumboreal. (Fig. 27.)

2. *A. alpestre* (Hoppe) Rylands.

Alpine Lady-fern

A. americanum (Butters) Maxon.

Rhizomes short, stout; fronds in a crown, 2-9 dm. long; stipes short, sparsely scaly, straw-colored from a dark base; blades oblong-lanceolate, narrowed toward the base; pinnae triangular-lanceolate, their rachises very narrowly winged; pinnules stalked, somewhat obliquely incised, the lower pinnatifid or pinnate; segments sharply toothed; sori round, small; indusia minute and evanescent. The general appearance is quite lacelike. Our form differs somewhat from the old world form and is the var. *americanum* Butters.

Alpine-arctic situations, southeastern Alaska—Calif. The entire species is circumboreal. (Fig. 28.)

3. Equisetaceae (Horsetail Family)

Rushlike plants with perennial, blackish, creeping rhizomes and hollow, jointed, simple or often much-branched stems bearing toothed sheaths at the joints. Spores borne in a terminal cone formed of verticels of peltate bracts bearing on the under surface a few sporangia which open on the inner side; spores uniform, provided with 4 hygroscopic bands; prothallia minute, green, lobed.

EQUISETUM L.

The only genus. (Latin, Equus, horse; and setum, bristle.)

1A. Stem annual, spike rounded at top, stomata scattered.

1B. Stems of 2 kinds, the fertile ones appearing earlier than the sterile ones.

1C. Fertile stem simple, soon withering. 1. *E. arvense*

2C. Fertile stem later producing branches.

1D. Branches simple. 2. *E. pratense*

2D. Branches compound. 3. *E. sylvaticum*

2B. Stems of one kind, branches simple or none.

1C. Center cavity small. 4. *E. palustre*

2C. Center cavity large. 5. *E. limosum*

2A. Stems perennial, evergreen; spike with a rigid tip; stomata in regular rows.

1B. Central cavity wanting, stems filiform. 6. *E. scirpiodes*

2B. Central cavity present.

1C. Stems slender, 5-10-grooved. 7. *E. variegatum*

2C. Stems medium, 8-12-grooved. 8. *E. alaskanum*

3C. Stems stout, 16-36-grooved. 9. *E. hiemale*

1. *E. arvense* L.

Common Horsetail

Rhizome slightly angled, felted, tuber-bearing; fertile stems erect, light-colored, 5-25 cm. tall; sheaths pale, loose, with 8-12 brownish, lanceolate teeth; spike ovoid, peduncled; sterile stems erect to decumbent, 1-5 dm. long, 6-14-furrowed, the numerous branches in verticels, 3-4-angled, solid; teeth of sheaths lanceolate, sharp-pointed. An extremely variable species. Seven varieties have been recognized in Alaskan material, but these do not seem to be permanent.

Common throughout the territory. Circumboreal, N. and S. Africa, Canaries. (Fig. 29.)

2. *E. pratense* Ehrh.

Thicket or Meadow Horsetail

Stems 1½-4 dm. long with 8-12 ridges, the fertile developing a few branches, spreading in age, the sterile with numerous long, simple branches; sheaths green, loose, the teeth lanceolate with dark middle; branches 3-ridged; teeth of the sheaths deltoid; cone peduncled; rhizome solid, acutely angled.

In woods, Bering Str. east and south. Circumboreal. (Fig. 30.)

3. *E. sylvaticum* L.

Wood Horsetail, Bottle-brush

Stems 1–5 dm. tall, 8–14-ridged, both fertile and sterile developing copious verticillate compound branches; sheaths loose, cylindrical or campanulate, the upper portion brown with more or less cohering teeth; primary branches 4–5-angled, the branchlets 3-angled, the sheaths with 3 divergent teeth.

In woods, Bering Str. east and south. Circumboreal. (Fig. 31.)

4. *E. palustre* L.

Marsh Horsetail

Rhizomes without felt or tubers; stems 2–9 dm. long, slender, the 5–10 angles of the stem with deep, winglike but rounded ridges; branches long, ascending, hollow, 5–7-angled; sheaths loose, widened upward, and the apices acute-subulate; spikes short-peduncled, terminating the stem with smaller ones terminating some of the branches.

Wet places, Bering Str. east and south. Circumboreal. (Fig. 32.)

5. *E. limosum* L.

Swamp Horsetail

E. fluviatile L.

Stems 5–15 dm. tall, 4–8 mm. thick, 10–30-grooved, with large central cavity, often simple, but more usually sparingly branched above with spreading or more often upcurved, 4–6-angled, simple branches; sheaths appressed with blackish, narrow, distinct teeth; cones short-peduncled.

In shallow water or swamps, Bering Sea east and south. Circumboreal. (Fig. 33.)

6. *E. scirpoides* Michx.

Little Horsetail

Stems tufted, simple or branched from the base, prostrate or weakly ascending, 5–15 cm. long, 6-ribbed by the deep grooving of the 3 angles, the ribs with a regular row of silica tubercles; sheaths loose, becoming black or dark brown, teeth 3, distinct, persistent, with a whitish border and a fragile subulate tip; cones 3–5 mm. long.

Damp situations, from above the Arctic Circle southward. Circumboreal. (Fig. 34.)

7. *E. variegatum* Schleich.

Northern Scouring-rush

Stems slender, tufted, 1–4 dm. long, 1–3 mm. thick, 5–10-grooved, the ridges bearing 2 lines of silica tubercles; sheaths loose, green below, dark above; teeth black with white border, persistent, with a filiform, deciduous tip; cones short-peduncled, 8–12 mm. long.

Throughout most of Alaska. Circumboreal. (Fig. 35.) A very small scirpoides-like form is var. *anceps* Milde.

8. *E. alaskanum* (A. A. Eaton), new comb.

Alaska Scouring-rush

E. variegatum var. *alaskanum* A. A. Eaton ex Gilbert.¹

Very similar to *E. variegatum* but much larger, growing up to at least 8 dm. long, the stems 2–4 mm. thick, 8–12-grooved.

Alaska Range and Bering Sea to Wash. Dr. Hultén has suggested

¹ Gilbert, B. D., List N. Amer. Pterid., p. 9, 1901.

that this type as well as similar forms elsewhere may have originated from the crossing of *E. hiemale* and *E. variegatum*. It is generally classified as a variety of *E. variegatum* but it appears to be quite distinct.

9. *E. hiemale* L.

Scouring-rush

Stems stiff, 5–15 dm. long, 5–10 mm. thick, unbranched, or with a few slender branches near the top, 16–36-ridged, rough with 2 rows of tubercles on the ridges; central cavity large; sheath with dark base and teeth with a light band between, the teeth adhering in groups by their pale, membranous margins; spike pointed, sessile or nearly so, 1–3 cm. long. Our form is the var. *californicum* Milde, which is more robust than the type.

Aleutian islands and central Alaska—Calif.—N. Mex. The entire species is circumboreal. (Fig. 36.)

4. LYCOPODIACEAE (Club-moss Family)

Low, evergreen, often mosslike, usually trailing plants with erect or ascending fruiting branches. Leaves very numerous, usually stiff, imbricate, 1-nerved, lanceolate or subulate; sporangia in the axils of the ordinary leaves, or more often in spikes at the base of modified leaves (sporophylls); spores minute and all of one kind; prothallia fleshy, subterranean.

LYCOPodium L.

Sporangia flattened, usually reniform, 1-celled; spores copious, sulfur yellow, inflammable. (Greek, wolf's foot.)

- 1A. Sporangia in the axils of ordinary leaves, not in spikes. 1. *L. selago*
- 2A. Sporangia borne in the axils of bracts arranged in spikes.
 - 1B. Spikes borne on bracteate pedicels more than 2 cm. long.
 - 1C. Branches flat, leaves in 4 rows. 2. *L. complanatum*
 - 2C. Branches terete, leaves in many rows. 3. *L. clavatum*
 - 2B. Spikes sessile or nearly so.
 - 1C. Aerial branches simple. 4. *L. inundatum*
 - 2C. Aerial branches mostly branched.
 - 1D. Aerial branches treelike. 5. *L. obscurum*
 - 2D. Aerial branches not treelike.
 - 1E. Leaves 4-ranked. 6. *L. alpinum*
 - 2E. Leaves 5-ranked. 7. *L. sitchense*
 - 3E. Leaves 8-ranked. 8. *L. annotinum*

1. *L. selago* L.

Fir Club-moss

L. porophyllum Lloyd and Underw.

Stem more or less curved below, erect or ascending above, 2–several times forked, forming tufts 3–20 cm. tall; leaves crowded, appressed or ascending, often spreading or reflexed near the base, narrowly triangular-

lanceolate or subulate, acute, usually entire, those bearing sporangia slightly shorter; plant usually producing gemmae. Very variable, the shade forms being dark green; the alpine and arctic form (var. *adpressum* Desv.) has closely appressed leaves and is yellowish-green in color.

Moist, rocky situations, throughout our range. Circumboreal. (Fig. 37.)

2. *L. complanatum* L.

Ground Cedar

Main stem creeping on or slightly below the surface of the ground; aerial branches yellowish-green, 4–40 cm. tall, usually much branched, the branches flattened, glaucous, with minute, decurrent, 4-ranked leaves, the lateral broad, the upper narrow, incurved, the lower small; peduncle 1–10 cm. long, bearing 1–4 spikes, each $1\frac{1}{2}$ –4 cm. long; sporophylls broadly ovate, acuminate, erose.

Bering Sea and central Alaska east and south. Circumboreal. (Fig. 38.)

3. *L. clavatum* L.

Running Pine

Main stem creeping, often 1–3 m. long; ascending branches 4–35 cm. tall, pinnately branched; leaves crowded, about 1×4 mm., many-ranked, linear-subulate, incurved-spreading, entire or denticulate, mostly bristle-tipped; peduncles 4–10 cm. long, branched at apex and bearing 2–4 spikes, the whorled or scattered bracts mostly bristle-tipped; sporophylls deltoid-ovate, abruptly acuminate, usually bristle-tipped, the margins membranous and erose.

Coniferous woods, coastal districts. Circumboreal. (Fig. 39.)

Var. *monostachyon* Grev. & Hook. has more incurved leaves about $3\frac{1}{4} \times 3\frac{1}{4}$ mm. and with more persistent bristles; peduncles 1–5 cm. long, bearing a single spike. Found mostly in the interior but collected near Seward and Juneau in the coast region. This variety is quite distinct so far as Alaska material is concerned, but there are connecting forms found elsewhere.

4. *L. inundatum* L.

Bog Club-moss

Plants small with simple or 1 or 2-forked, short-creeping leafy stems; fertile stems erect, 1–8 cm. tall; leaves of the creeping stems linear-lanceolate and upcurved; leaves of the ascending branches spreading; spike solitary; sporophylls similar to the leaves but with wider ovate base, spreading, usually entire.

Growing in mud, Wrangell—Ore.—Newf.—N. J. Also in Europe and eastern Asia. (Fig. 40.)

5. *L. obscurum* L.

Ground-pine

L. dendroideum Michx.

Main stem creeping underground; aerial branches treelike, 10–35 cm. tall with bushy branches; leaves 8-ranked on the lower branches, 6-ranked on the terminal ones, narrowly lanceolate, spreading but curved upward and usually twisted, acute or mucronate; sporophylls broadly

ovate, abruptly acuminate, with scarious, erose margins. Our plant is usually classified as var. *dendroideum* (Michx.) D. C. Eat.

Woods, Alaska distribution scattered, Aleutian Islands, central and southeastern Alaska—Baffinland—Ala.—S. Dak.—Wash. Much used by florists. (Fig. 41.)

6. *L. alpinum* L.

Alpine Club-moss

Main stem creeping on or near the surface of the ground, aerial branches ascending, 2½–11 cm. tall, repeatedly branched, the sterile branches flat with 4-ranked leaves; fertile branches terete with subulate leaves; spikes sessile or nearly so; sporophylls ovate, acute, erose; spores reticulated. Alaska material approaches *L. complanatum* on one hand and *L. sitchense* on the other.

Seward peninsula east and south. Circumboreal. (Fig. 42.)

7. *L. sitchense* Rupr.

Alaska Club-moss

L. sabinaefolium Willd. var. *sitchense* (Rupr.) Fern.

Main stem creeping on or near the surface of the ground, aerial branches several times dichotomous, forming compact tufts 4–8 cm. tall with longer projecting fertile branches; branches terete; leaves of the branchlets 5-ranked, appressed or somewhat spreading, linear, thick, entire, acute; spikes usually sessile, sometimes short-stalked; sporophylls broadly ovate, long-acuminate or subulate, greenish, with scarious, more-or-less erose margins.

Alaska—Lab.—N. Y.—Ore. (Fig. 43.)

8. *L. annotinum* L.

Stiff Club-moss

Main stem creeping on or in moss, up to 4 m. in length; aerial branches 4–35 cm. tall, usually forked 1–4 times; leaves linear-lanceolate, usually serrulate, tipped with a rigid point, spreading or rarely reflexed, upcurved at apex; sporophylls broadly ovate, abruptly acuminate-attenuate. The var. *pungens* (LaPylaie) Desv. is an arctic-alpine form with small, entire, very acute, curved ascending leaves.

Woods, bogs, and alpine meadows, southward from about 68 degrees. Circumboreal. (Fig. 44.)

5. SELAGINELLACEAE (Little Club-moss Family)

Small, leafy, mosslike plants with branching, often prostrate stems and scalelike, 4–6-ranked leaves. Sporangia solitary in the axils of leafy bracts, some containing small, pollen-like spores (microspores), others containing large spores (macrospores) with a roundish base and a triangular-pyramidal apex.

SELAGINELLA Beauv.

Characters of the family. (Diminutive of Selago, ancient name of some Lycopodium).

Bracts thin, spreading, similar to the leaves. 1. *S. selaginoides*
Bracts broader, in quadrangular spikes. 2. *S. sibirica*

1. *S. selaginoides* (L.) Link.

Low Selaginella

Sterile stems prostrate, soft and usually slender, the fertile erect or ascending, 3–8 cm. tall; leaves lanceolate, acute, spreading, sparsely spinulose-ciliate, those of the spike longer, ascending, strongly ciliate; macrospores large, individually visible to the naked eye.

Aleutians, Bering Str., and Wiseman southward and eastward. Circumboreal. (Fig. 45.)

2. *S. sibirica* (Milde) Hieron.

Northern Selaginella

S. schmidtii Hieron.

Stems creeping and rooting, forming a dense mat; fertile branches ascending or erect, 1–5 cm. tall; leaves densely imbricated, those of the stem linear-oblong, stiff, about 1½ mm. long with deciduous apical awns about ½ mm. or more long, the margins minutely ciliate; spikes sharply 4-angled, about 1½ mm. thick, the bracts ovate-lanceolate, about 2 mm. long, with short awn.

Dry rocky situations, interior Alaska and Yukon. Also eastern Asia. (Fig. 46.)

6. ISOETACEAE (Quillwort Family)

Small aquatic or marsh plants with short cormlike stem and many crowded subulate or nearly filiform leaves bearing sporangia embedded in their bases. Spores of two kinds, the inner sporangia bearing the microspores, the outer leaves enclosing sporangia with macrospores.

ISOETES L.

The only genus. (Greek, equal at all seasons.)

I. braunii Durieu.

Braun's Quillwort

I. braunii Durieu var. *maritima* (Underw.) Pfeiff.*I. echinospora* Durieu var. *truncata* Eaton.

Leaves 7–20, erect or spreading, tapering, 2½–5 cm. long; macrospore nearly ½ mm. in diameter, densely covered with broad, often retuse spinules; microspores smooth.

Coastal districts, Aleutians to southeastern Alaska—Greenl.—N. J.—Colo.—Calif. (Fig. 47.)

PHYLUM SPERMATOPHYTA (Seed-bearing Plants)

Plants producing seeds containing young plants in a dormant condition until germination. This seed is the result of the fertilization of the egg-cell of the ovule by a sperm-cell from a pollen-grain. The grains of pollen correspond to the microspore of the heterosporous Pteridophytes while the macrospore is contained within the ovule.

There are probably 150,000 species in existence, and they form the predominant vegetation of the present geological epoch. The diversity and number of species grow progressively greater from the polar regions to the tropics.

Ovules and seed borne on the face of a scale, not enclosed.....	Class 1. <i>Gymnospermae</i>
Ovules and seed contained in a closed cavity (ovary).....	Class 2. <i>Angiospermae</i>

CLASS 1. GYMNOSPERMAE

Ovules naked, borne on the flat surface of a scale which does not infold to form an ovary, such scale sometimes apparently wanting. Pollen grains dividing at maturity into two or more cells, one of which gives rise to the pollen-tube.

An ancient group which reached its peak in the Triassic geological time; now represented by scarcely 500 species of wide geographic distribution, some of which are of great economic value. Most of the lumber used by mankind is furnished by trees of this group. Only one order (*Coniferales*) of this class is represented in our area. There are two families.

Ovulate flowers without carpellary scales.	Fam. 1. <i>Taxaceae</i>
Ovulate flowers with carpellary scales.	Fam. 2. <i>Pinaceae</i>

1. TAXACEAE (Yew Family)

Evergreen trees or shrubs with linear leaves and dioecious flowers which are axillary and surrounded by bud-scales; the staminate globular, and formed of a few naked stamens; the fertile consisting of an erect ovule developing a fleshy coating.

TAXUS (Tourn.) L.

Branches horizontal or drooping with linear or lanceolate, flat, keeled leaves, revolute on the margins, and persisting 4-5 years. (The classical name.)

T. brevifolia Nutt. Western Yew

In Alaska reduced to a small tree or shrub not over 10 m. tall. Leaves yellowish-green, 12-16 mm. long, 1-2 mm. wide, acute, 2-ranked by a twist of the flattened and decurrent petiole; fruit red, drupelike.

Extreme southeastern Alaska—Mont.—Calif.

2. PINACEAE (Pine Family)

Resinous trees or shrubs with linear, needle-like or scalelike leaves. Flowers usually monoecious, in scaly aments, the fertile ones becoming cones or berry-like; ovules 2 or more at the base of each fertile scale. All are evergreen except *Larix*. Our species naturally fall into two groups or subfamilies.

1A. Scales of the fertile cones few, opposite (*Cupresseae*).

1B. Fruit berry-like1. *Juniperus*

2B. Fruit a cone.

1C. Cone ovoid, its scales oblong.....2. *Thuja*

2C. Cone globose, its scales peltate.3. *Chamaecyparis*

2A. Scales of fertile cones many, alternate (*Abieteeae*)

1B. Leaves in clusters of 2 or more.

1C. Leaves evergreen.4. *Pinus*

2C. Leaves deciduous5. *Larix*

2B. Leaves solitary.

1C. Cones erect.6. *Abies*

2C. Cones pendent.

1D. Leaves flat, blunt.7. *Tsuga*

2D. Leaves quadrangular or thick, acute.8. *Picea*

1. JUNIPERUS (Tourn.) L.

Aromatic trees and shrubs with subulate or scalelike sessile leaves; staminate aments oblong or ovoid, anthers 2-6-celled, each 2-valved; fertile aments of 3-6 fleshy coalescent scales, becoming berry-like, blue fruits, each with 1-6 wingless bony seeds. (The classical name).

Leaves all subulate.1. *J. communis*

Leaves mostly scalelike on mature plants.....2. *J. horizontalis*

1. *J. communis* L. var. *montana* Ait.

Low Juniper

J. sibirica Burgsd.

J. nana Willd.

J. communis L. var. *sibirica* Rydb.

A depressed or trailing alpine-arctic shrub forming patches up to 3 m. in diameter. Leaves in whorls of 3, ascending or spreading, 6-10 mm. long, rigid, pungently acute, shining, keeled or strongly convex below, grooved above; staminate aments ovate, 3-6 mm. long; berries globose, 7-9 mm. broad, blue, covered with a white bloom. In some more southern regions this low-growing form intergrades with the upright type.

Throughout most of Alaska. Circumboreal. (Fig. 48.)

2. *J. horizontalis* Moench.

Creeping Juniper

J. prostrata Pers.

A prostrate shrub with the stem often rooting. Leaves of young plants subulate, those of the mature stem scalelike, 4-ranked, acute or acuminate; fruits blue, somewhat glaucous, 7-9 mm. in diameter, on short recurved pedicel-like branches.

Southeastern interior Alaska—Lab.—Newf.—Maine—Iowa—Colo. (Fig. 49.)

2. THUJA L.

Forest trees with frondlike branches, the leaves small and scalelike, appressed, imbricated, opposite, 4-ranked; staminate aments ovate, with 4-6 peltate scales, each bearing 2-4 globose anther-sacs; ovulate aments oblong with 8-12 scales; cones pendulous, their scales thin and flexible. (Ancient name.)

T. plicata D. Don.

Western Red Cedar, Giant Cedar

T. gigantea Nutt.

A large tree with thin, fibrous bark; branchlets bright green and shining above, paler beneath; leaves ovate, short-pointed, about 3 mm. long, obscurely glandular-pitted; cones clustered near the ends of the branches, soon reflexed, 10–14 mm. long; scales leathery. A valuable tree, the wood is soft, brittle, aromatic, light reddish-brown and very durable.

From about 57 degrees N.—Mont.—Calif. (Fig. 50.)

3. CHAMAECYPARIS Spach.

Resembling *Thuja* in general appearance; bark thin, scaly; branchlets 2-ranked in a horizontal plane; leaves scalelike, ovate, acuminate, opposite in pairs; staminate aments oblong; ovulate aments globose, the mature cones with woody, peltate scales, each with a central projection. (Greek, meaning low cypress.)

C. nootkatensis (Lamb.) Spach.

Yellow Cedar, Alaska Cypress

A medium-sized tree with drooping branchlets; leaves hardly glandular, convex or ridged on the back, pointed, appressed except on vigorous shoots; staminate aments about 4 mm. long; cones subglobose, 10–12 mm. broad. The wood is aromatic, sulfur-yellow, fine-grained, and durable.

Along the coast, Prince William Sound to Ore. (Fig. 51.)

4. PINUS (Tourn.) L.

Trees or shrubs with scalelike deciduous primary leaves and needle-like secondary leaves, the secondary leaves borne in clusters of 2–5 terminating short rudimentary branchlets in the axils of the primary leaves and comprising the ordinary foliage which persists for 2–8 years; staminate aments clustered at the base of the seasons growth, forming a distinct zone which remains naked after the aments have fallen; ovulate aments solitary or clustered, borne on twigs of preceding season, composed of many scales and developing into cones the second season, the scales elongating and becoming woody; seeds 2, at the base of the scales, winged above. (The classical Latin name.)

P. contorta Loud.

Lodgepole or Tamarack Pine, Scrub Pine

P. murrayana Balf.

Usually a low scrubby tree or shrub growing in and around muskeags in the coast region. Leaves in 2's, 3–5 cm. long; staminate aments orange-red, about 8 mm. long; cones oblique-ovoid, 3–5 cm. long, usually persisting for several years; wood hard, light reddish-brown, coarse-grained.

Glacier Bay—Calif. The form in Yukon Ter. is the var. *latifolia* Engelm. (Var. *murrayana* Engelm.), an upright-growing, slender tree up to 25 m. tall and distributed in the mountains from the upper Yukon Valley—Colo.—Calif. (Fig. 52.)

5. *LARIX* (Tourn.) L.

Trees with small, linear, deciduous leaves in fascicles on short, lateral, scaly, budlike branchlets; staminate aments from leafless buds; the ovulate from buds leafy at base, red; cones erect, ovoid, small, with thin scales. (Ancient name.)

L. laricina (DuRoi) Koch.

American Larch, Tamarack

L. americana Michx.

L. alaskensis Wight.

A small tree, the trunk seldom more than 15 cm. in diameter; leaves 10–20 in a cluster, 15–25 mm. long; cones ovoid, 10–18 mm. long; scales 12–18, suborbicular, thin; wood hard, strong, light brown, resinous, durable.

Wet situations, interior Alaska, Bering Sea—Lab.—Newf.—Mass.—Ill. (Fig. 53.)

6. *ABIES* (Tourn.) Hill.

Trees with linear, flat, scattered leaves spreading and twisting so as to appear 2-ranked; except on fruiting branches where the leaves are 4-sided and curve upward; staminate aments axillary; ovulate aments lateral; cones erect, subcylindrical or ovoid, their scales deciduous from the persistent axis, thin, incurved at the broad apex. (Ancient name.)

Leaves with stomata on both sides. 1. *A. lasiocarpa*

Leaves with stomata on lower surface only. 2. *A. amabilis*

1. *A. lasiocarpa* (Hook.) Nutt.

Alpine Fir

A small or medium-sized tree, or at timberline scrubby, with smooth bark except on the oldest and largest trees; branchlets rusty-pubescent; leaves rounded or notched at the apex, grooved on upper side, 20–35 mm. long; scales fan-shaped; wood fine-grained, soft, weak.

Copper river district and southeastern Alaska—Alta.—N. Mex.—Ore. (Fig. 54.)

2. *A. amabilis* (Dougl.) Forbes.

Silver Fir, Lovely Fir

A large tree with smooth, gray, white-splotted bark; leaves grooved and green above, whitish beneath, 2–3 cm. long, recurved on the margins, obtuse or notched at apex, erect on the branches by the recurving of those on the lower side; cones oblong, 9–15 cm. long; wood pale brown, hard but weak.

Coast ranges, extreme southeastern Alaska—Ore.

7. *TSUGA* Carr.

Trees with slender, horizontal, or drooping branches; leaves linear, short-petioled, scattered, appearing 2-ranked by the spreading and twisting of the petioles, jointed to very short sterigmata and falling away on drying; staminate aments axillary, subglobose to ovate; ovulate aments terminal, erect; cones pendulous; scales thin. (Name Japanese.)

- Cones small, about 2 cm. long1. *T. heterophylla*
 Cones larger, about 5 cm. long2. *T. mertensiana*

1. *T. heterophylla* (Raf.) Sarg.

Western Hemlock

A large forest tree up to 60 m. tall and 1½ m. in diameter; branchlets yellowish, pubescent; leaves flat, rounded at the apex, deeply grooved, 8–20 mm. long; staminate aments yellow; ovulate aments purple; cones 16–22 mm. long; scales puberulent; wood pale yellowish-brown, light, hard and strong. Comprises fully 70 per cent of the forest stand in southeastern Alaska.

Kenai Peninsula—Ida.—Ore. (Fig. 55.)

2. *T. mertensiana* (Bong.) Sarg.

Mountain Hemlock

Hesperopeuce mertensiana (Bong.) Rydb.

A small or medium-sized tree up to 30 m. tall and 9 dm. in diameter, but a mere shrub on muskeags and at timberline; leaves convex or keeled below, grooved above, narrowed toward the base, rounded at the apex, 12–22 mm. long; staminate aments purplish; ovulate aments deep purple; cones sessile, 4–6 cm. long; wood fine-grained, soft, and light.

Cook Inlet—Ida.—Mont.—Calif. (Fig. 56.)

8. *PICEA* Link.

Forest trees with whorled branches; leaves linear, short, horny-tipped and spreading in all directions, persisting for several seasons, jointed at the base to short persistent sterigmata, falling away in drying; staminate aments axillary; ovulate aments terminal, erect; cones pendulous, their scales numerous, thin, obtuse, persistent. (Name ancient.)

1A. Cones 1½–3 cm. long, persisting for several years 1. *P. mariana*

2A. Cones 4–10 cm. long, falling off at maturity.

1B. Leaves quadrangular.2. *P. glauca*

2B. Leaves rather flat.3. *P. sitchensis*

1. *P. mariana* (Mill.) B.S.P.

Black Spruce

A small tree, often scrubby, with pubescent branchlets; leaves stout, generally curved, glaucous, quadrangular, with blunt tip, 6–10 mm. long; cones oval or ovoid; the scales usually with slightly erose margins.

Muskeags and hillsides, southwest Alaska to north of the Arctic Circle—Ungava Bay—Newf.—N. Car.—Wis.—Alta. (Fig. 57.)

2. *P. glauca* (Moench) Voss.

White Spruce

P. canadensis (Mill.) B.S.P.

A medium-sized tree up to 28 m. tall and nearly 1 m. in diameter; branchlets glabrous; leaves rather slender, acute, 12–20 mm. long, bluish-green with more or less bloom; cones cylindric or oblong-cylindric, 3–6 cm. long, the scales thin, entire.

Southwestern Alaska—Noatak River—Ungava Bay—Newf.—Maine—Wis.—Alta. Larger trees of this species furnish most of the lumber sawed in interior Alaska. (Fig. 58.)

3. *P. sitchensis* (Bong.) Carr.

Sitka Spruce

Our largest tree, reaching a height of 50 m. and a diameter of $2\frac{1}{2}$ m. or more; branchlets glabrous; leaves acute or acuminate, 15–25 mm. long, keeled on upper surface, rounded or slightly keeled on lower surface, with 2 narrow bands of whitish stomata above and 2 wide bands below; staminate aments dark red; cones cylindrical or narrowly oblong-oval, 5–10 cm. long; scales thin, denticulate above the middle.

Coast region, Kodiak Island and Cook Inlet—Calif. Our most valuable tree. Beside furnishing construction lumber and wood-pulp it is used in airplane construction and for piano sounding boards. (Fig. 59.)

PLATE I

- FIG. 1. *Botrychium lunaria*
2. *Botrychium boreale*
3. *Botrychium lanceolatum*
4. *Botrychium silaifolium*
5. *Blechnum spicant*
6. *Struthiopteris filicastrum*
7. *Cryptogramma acrostichoides*
8. *Cryptogramma stelleri*
9. *Adiantum pedatum*
10. *Pteridium aquilinum lanuginosum*
11. *Polypodium vulgare occidentale*
12. *Woodsia glabella*

PLATE I

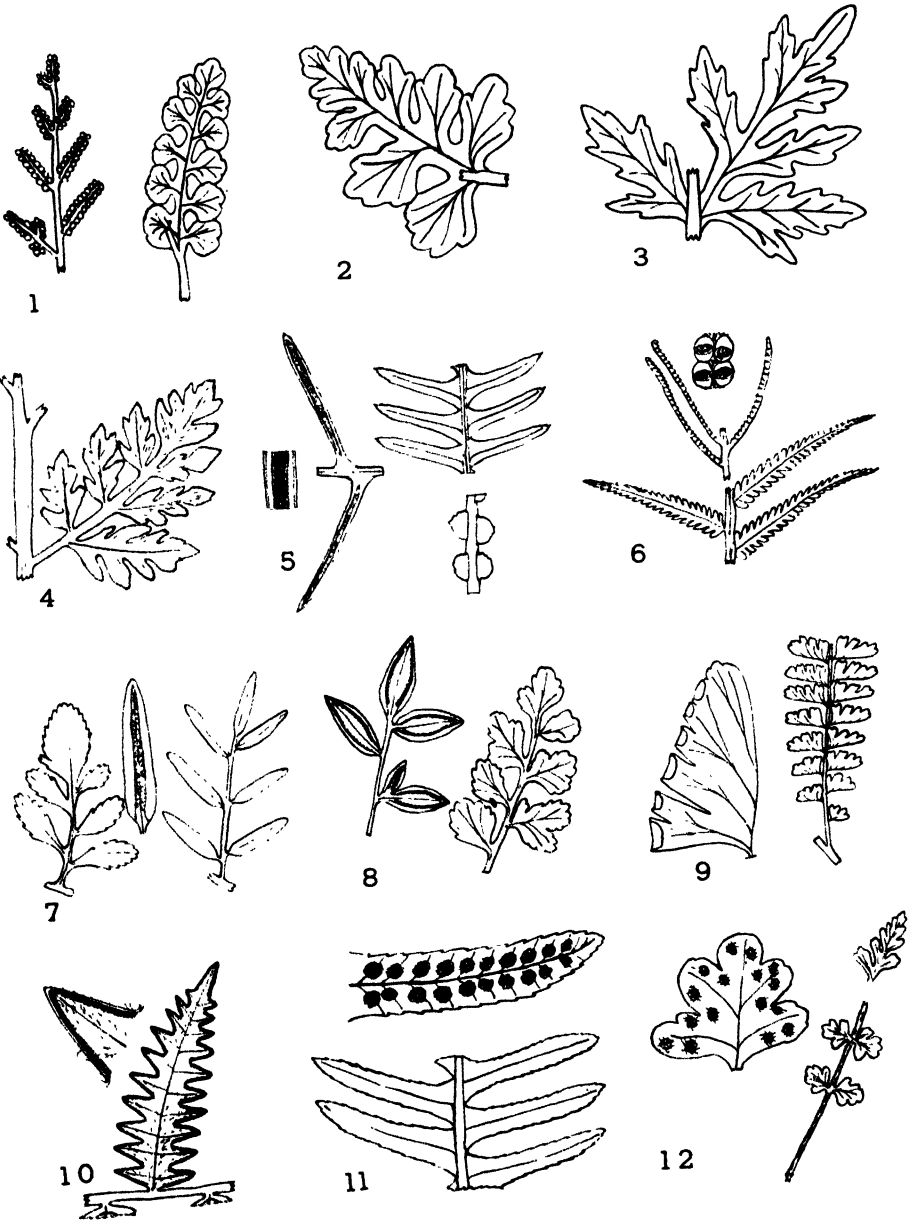


PLATE II

- FIG. 13. *Woodsia alpina*
14. *Woodsia ilvensis*
15. *Cystopteris fragilis*
16. *Cystopteris montana*
17. *Polystichum lonchites*
18. *Polystichum munitum*
19. *Polystichum braunii*
20. *Dryopteris phegopteris*
21. *Dryopteris linnaeana*
22. *Dryopteris fragrans*
23. *Dryopteris oreopteris*
24. *Dryopteris austriaca*

PLATE II

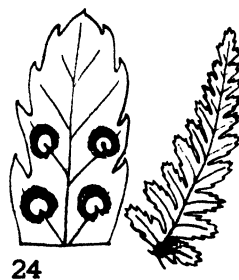
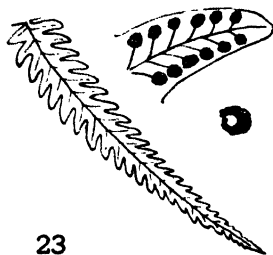
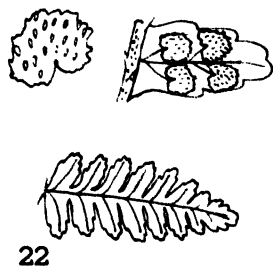
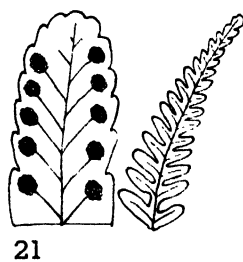
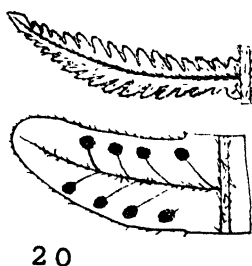
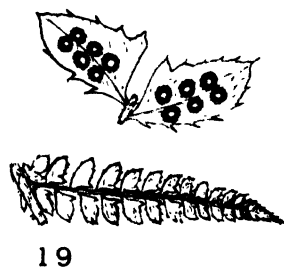
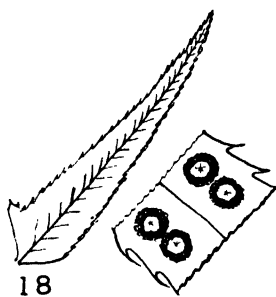
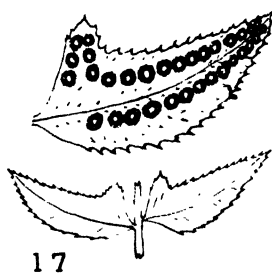
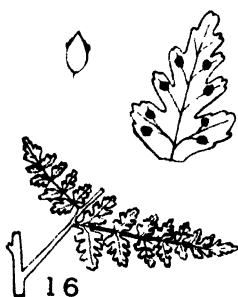
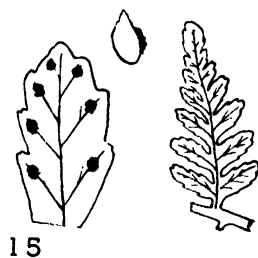
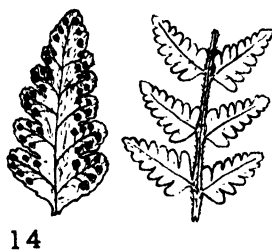


PLATE III

- FIG. 25. *Asplenium trichomenes*
26. *Asplenium viride*
27. *Athyrium filix-femina* var.
28. *Athyrium alpestre* var.
29. *Equisetum arvense*
30. *Equisetum pratense*
31. *Equisetum sylvaticum*
32. *Equisetum palustre*
33. *Equisetum limosum*
34. *Equisetum scirpioides*
35. *Equisetum variegatum*
36. *Equisetum hiemale* var.

PLATE III

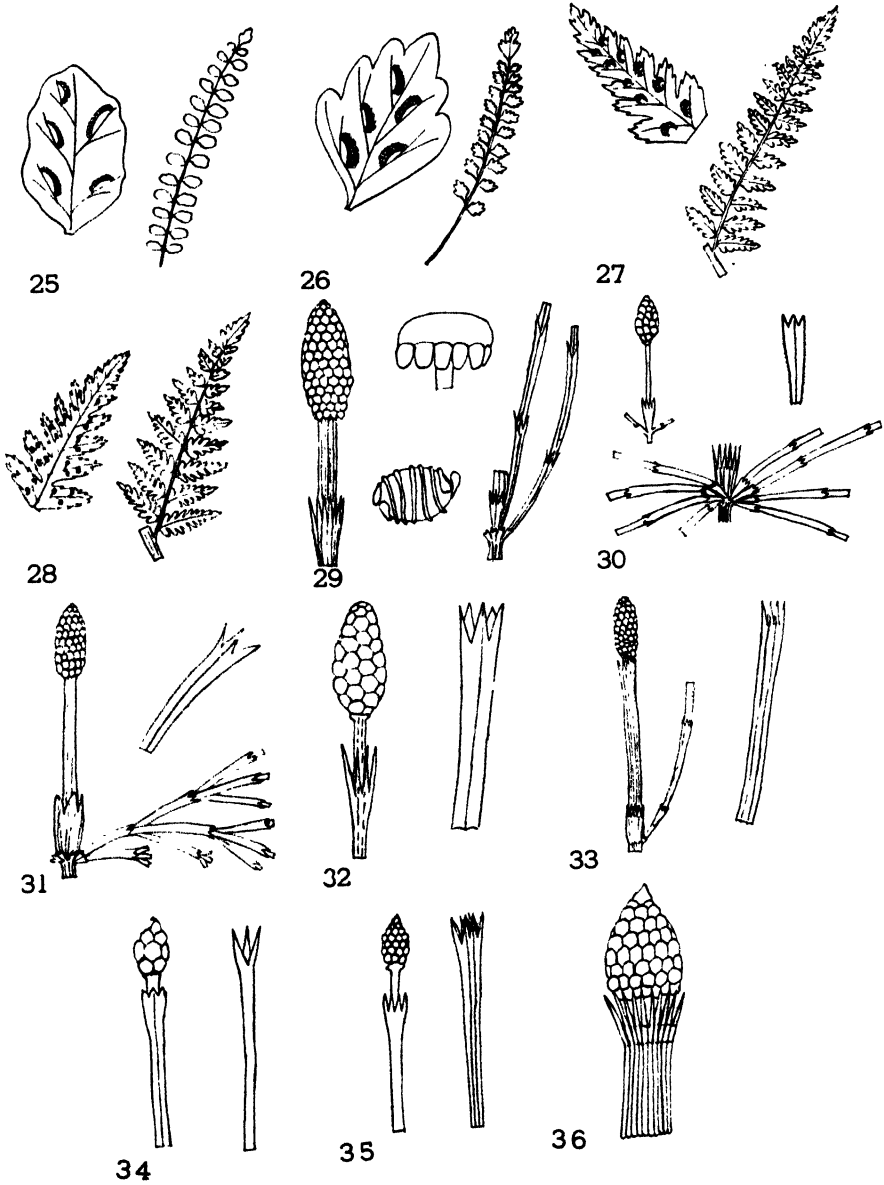


PLATE IV

- FIG. 37. *Lycopodium selago*
38. *Lycopodium complanatum*
39. *Lycopodium clavatum*
40. *Lycopodium inundatum*
41. *Lycopodium obscurum*
42. *Lycopodium alpinum*
43. *Lycopodium sitchense*
44. *Lycopodium annotinum*
45. *Selaginella selaginoides*
46. *Selaginella sibirica*
47. *Isoetes braunii*
48. *Juniperus communis* var. *montana*

PLATE IV

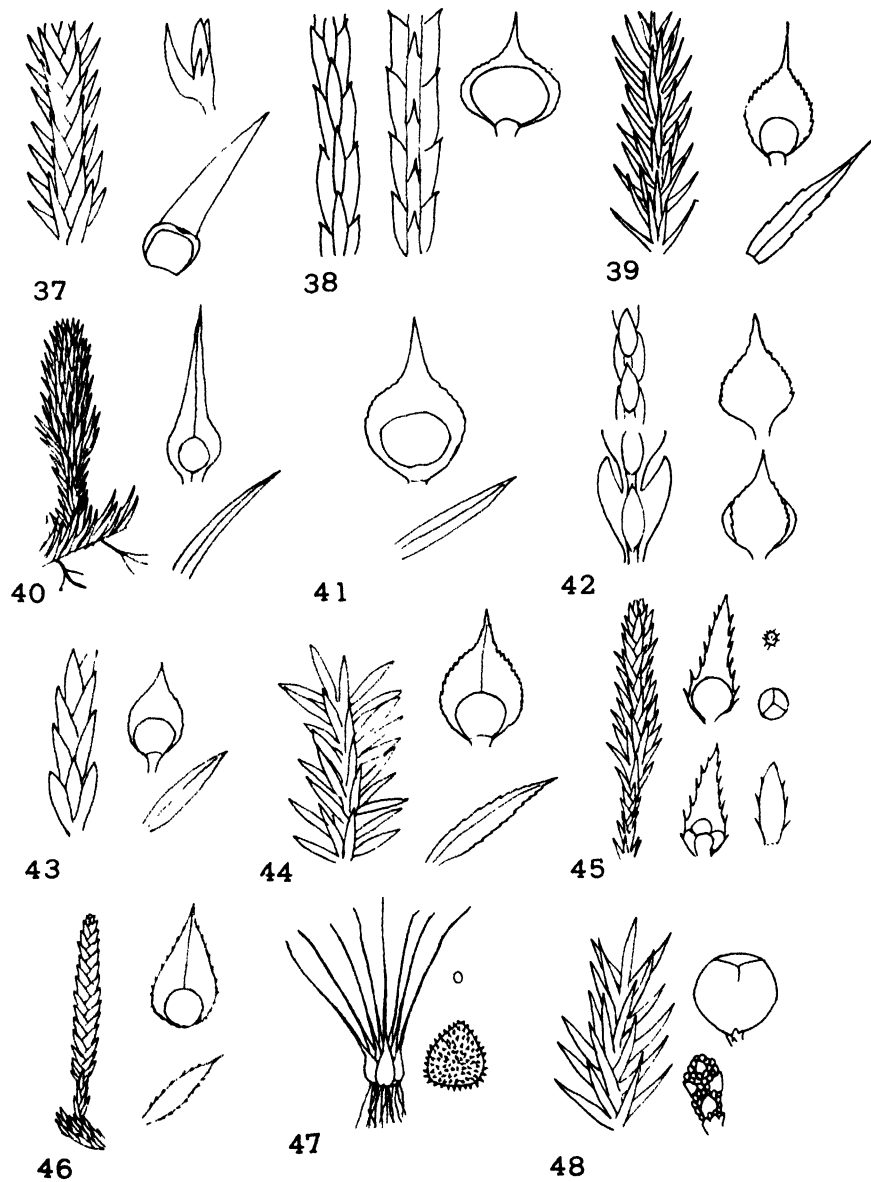
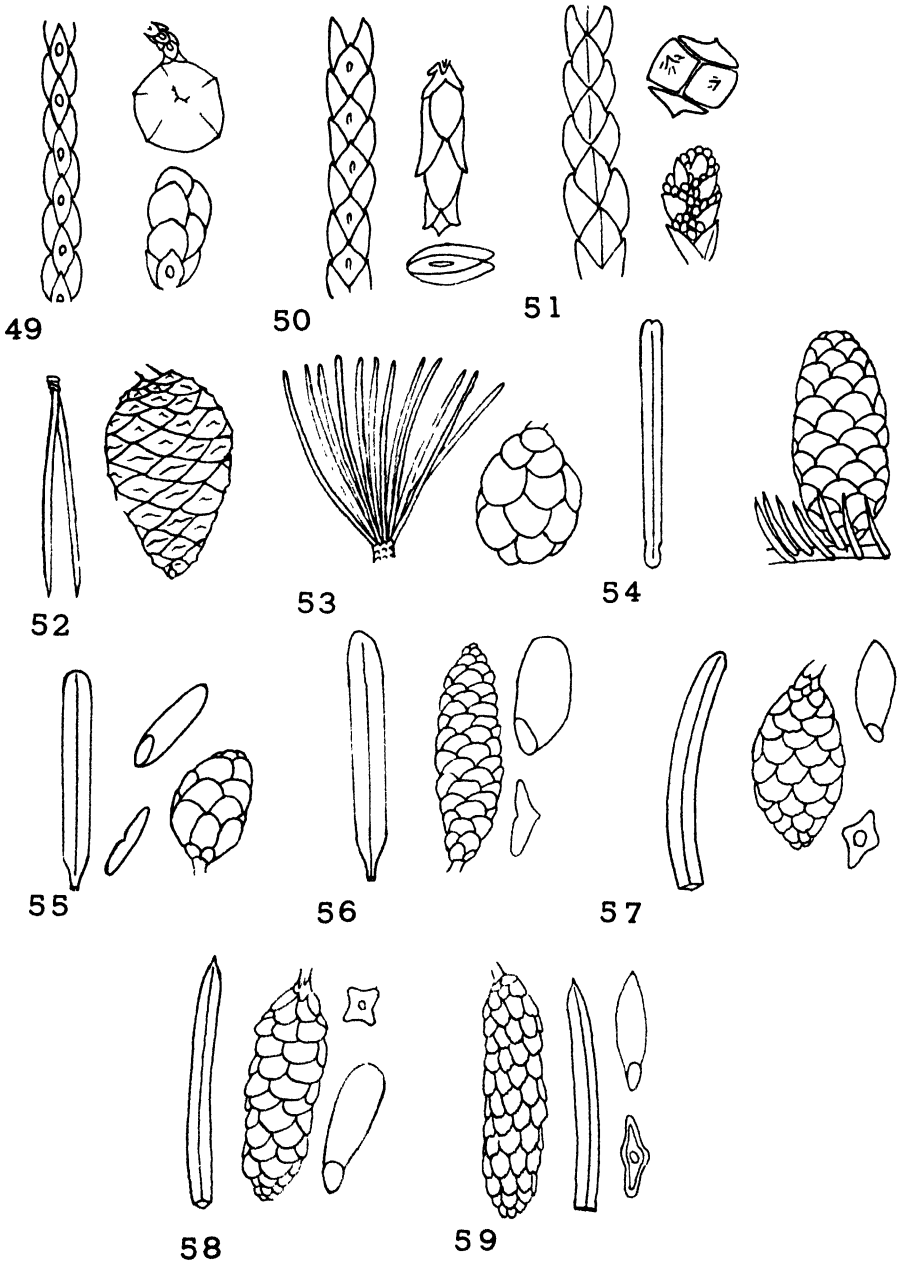


PLATE V

- FIG. 49. *Juniperus horizontalis*
50. *Thuja plicata*
51. *Chamaecyparis nootkatensis*
52. *Pinus contorta*
53. *Larix laricina*
54. *Abies lasiocarpa*
55. *Tsuga heterophylla*
56. *Tsuga mertensiana*
57. *Picea mariana*
58. *Picea glauca*
59. *Picea sitchensis*

PLATE V



THE COCCIDIA OF WILD RABBITS OF IOWA

II. EXPERIMENTAL STUDIES WITH *EIMERIA NEOLEPORIS* CARVALHO, 1942¹

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During the author's experiments with coccidia of wild rabbits in Iowa, the most complete studies were made with *E. neoleporis*, because it was able to grow in the tame rabbit. Experiments were carried on to observe its behavior, life cycle, biometrical or physiological changes, immunity relationships, etc., in the latter host. Comparative studies in both hosts were also performed.

Results obtained led the author to believe that sooner or later *E. neoleporis* will extend its host range. One is also led to contemplate its evolutionary derivation, perhaps from *Eimeria leporis* of European hares. Speciation in this case has been accompanied by morphological and physiological manifestations (in the micropyle, residual body, intestinal localization, etc.), and indications are that it is still going on, while the presumed original source has remained unchanged.

Quantitative counts were made by Becker and Derbyshire's (1937) method. The schizogonic cycle of *E. neoleporis* was studied in the tame rabbit. Heavy dosages, as high as 600,000 oocysts, were given to 18-day-old rabbits, free from coccidia. A rabbit was killed every 48 hours until completion of the life cycle. Localization of the infection was ascertained by killing a rabbit and sectioning different parts of the intestine 10 days after the experimental infection was induced. Macroscopic examination and intestinal smears are of great help in this procedure.

The histological technique employed was that of Roudabush (1937). Both Zenker's and Bouin's solutions were used as fixatives. Sections were stained with Goldhorn's polychrome methylene blue and eosin or Delafield's hematoxylin. Smears were made systematically, observed when still fresh, and after fixation and staining. Schaudinn's solution and absolute alcohol were used for fixatives, and Goldhorn's polychrome methylene blue, Delafield's hematoxylin, and iron-hematoxylin were chosen for stains.

The generations of merozoites, the sporozoites, and the gametes

¹ Journal paper No. J-1075 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 570. The Fish and Wildlife Service (U. S. Dept. of the Interior), Iowa State College, Iowa State Conservation Commission, and American Wildlife Institute cooperating. Taken from a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirement for the degree, Doctor of Philosophy. Doctoral thesis No. 689A. Part II.

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were studied in fresh and fixed conditions. Measurements and drawings were taken with the aid of a camera lucida. The determination of the number of generations of schizonts was the same as that adopted by Roudabush (1937).

Experiments on immunity to *E. neoleporis* were made with groups of young tame rabbits (entirely free from coccidia before the experimental infection) and with adult rabbits previously infected with other species of *Eimeria*.

ENDOGENOUS CYCLE

Developmental stages of *E. neoleporis* were found to be localized in the apical process of the cecum and in the ileo-cecal valve of the experimentally infected tame rabbits. This localization is the same as that reported by Waller and Morgan (1941) for *E. leporis* (presumably *neoleporis*) in cottontails. Endogenous stages of *E. neoleporis* have the same localization in both cottontails and tame rabbits. The infection is easily detected macroscopically after the fifth day when the apical process of the cecum becomes whitish, enlarged, and shows hyperemic blood vessels. The usual corduroid aspect characteristic of coccidia lesions was distinct on and after the sixth day. The ileo-cecal valve is apparently attacked more during the later stages, and cases were seen in which it became so enlarged as almost to obstruct the passage of intestinal contents to the large intestine and cecum.

During the first 3 days of oocyst elimination the cecal contents, large intestine, and even pellets are hemorrhagic. Before this period the lumen is filled with a caseous material in which merozoites and leucocytes are abundant. The epithelial layer is badly damaged during the oocyst discharge. Intense desquamation of epithelial cells and hemorrhage results, and leucocytes, mainly lymphocytes and eosinophils, are present in excessive numbers in the parasitized area. Other lesions are the same as known for other previously studied species.

As previously mentioned, the prepatent period for the infection averages 12 days, while the patent period is about 10 days. Sporozoites (Pl. I, Fig. 1) were detected in the cecal lumen until the fourth day after administration of the oocysts. This proves that they may have a prepatent period of from 1 to 4 days, thus causing a superposition in the development of not only the first, but subsequent generations of schizonts. Sporozoites recovered from the cecal lumen had the same morphology as those still within the sporocyst. The refractive granules acquired a reddish color when stained with Goldhorn's polychrome methylene blue. The average dimensions were 14.3 by 4.5 μ .

The first schizogonic generation is produced by sporozoitic invasion of the epithelial cells of the apical process, mainly those localized at the bottom of the crypts. Young schizonts (Pl. I, Fig. 2) are found in the epithelial layer, and later stages, principally mature schizonts (Pl. I, Fig. 3), in the tunica propria of the mucous membrane. This migration

of infected cells agrees with that described by Matsubayashi (1934) and Cheissin (1940) for certain other species in rabbits.

No attempt was made to measure either growing schizonts or mature schizonts, in which the size depends on the fluctuating number of merozoites. The mature schizonts contain about 43 to 48 merozoites (Pl. I, Fig. 4) and a central residual mass. The escape of merozoites into the intestinal lumen is begun by the end of the fifth day. Merozoites of the first generation (Pl. I, Fig. 5) resemble a *Leishmania* in form, with the nucleus nearer to the blunt end. They average 20.5 by 2.8 μ in size. Merozoites of this type were observed in smears until the eighth day, indicating a period of 4 days for completion of the first generation of schizonts.

The invasion of other epithelial cells by first-generation sporozoites gives rise to the second schizogonic generation, which attains its full development on the seventh day. Growing schizonts of this generation (Pl. I, Fig. 6) can be seen on the sixth day. Mature schizonts show from 59 to 72 merozoites (Pl. I, Fig. 7) which, after breaking from the cells and falling into the lumen, average 25.7 by 1.5 μ . These merozoites are Crithidia-like in form, with one nucleus and 4 to 5 siderophilic granules (Pl. I, Fig. 8). Intestinal smears revealed their presence in the lumen until the ninth day.

Schizonts of the third generation (Pl. I, Figs. 9 and 10) fully mature on the ninth day. Two types of schizonts seem to be present in this generation; a smaller (Pl. I, Fig. 12), with an average of 14 merozoites and a larger (Pl. I, Fig. 11), with 60 to 86 merozoites. Merozoites of the smaller schizont (Pl. I, Fig. 13b) measure 18.0 by 3.5 μ , whereas those of the larger (Pl. I, Fig. 13a) measure 31.5 to 1.5 μ . The presence of two types of merozoites in this generation suggests that while one (smaller) continues the asexual cycle the other (larger) is destined to give rise to gametocytes, which can be seen from the tenth day henceforth until the end of the infection. This assertion is based on the fact that a somewhat different type of merozoite was seen in the smears of the twelfth day, although no mature schizonts could be detected after the ninth day (probably due to the small number of them). This would bring the number of schizogonic generations to four, of which three are easily and distinctly recognizable.

Gametocyte formation starts on the tenth day. Growing macro- and microgametocytes reach full development 2 days later. Differentiation of gametocytes can be detected only after the eleventh day, since the young gametocytes are indistinguishable.

The microgametocyte nuclei divide several times (Pl. I, Fig. 14), forming several spherical black corpuscles which later become slightly elongated and finally comma-shaped (Pl. I, Fig. 15a). On the twelfth day these microgametes can be seen free in the tissue and a few in the intestinal lumen. Two flagella are present at the anterior end. The size of the body is about $3.5 \times 0.5 \mu$ and of the flagella 0.6 μ long (Pl. I, Fig. 15b).

Macrogametocytes (Pl. I, Fig. 16) are found in the apical process of the cecum and in the ileo-cecal valve, where they occur in large numbers forming several "oocyst nests" (Pl. I, Fig. 17). They can be differentiated from microgametocytes by the presence of both plactic and siderophilic granules, whereas the latter possess only siderophilic granules. With Delafield's hematoxylin the microgametocytes appear as small black dots, whereas in the macrogametocytes there are plactic granules of a somewhat dull-yellow color associated with small blue to black granules. Of these the dull-yellow granules are the more numerous. Both migrate to the periphery of the macrogametocyte (Pl. I, Fig. 18) and give rise to the outer membranes of the oocyst. Young oocysts (Pl. I, Fig. 19) can be seen by the end of the eleventh day.

The invasion of cells by several macrogametocytes heretofore mentioned by several authors was verified. As many as 18 young macrogametocytes were seen within one cell or "oocyst nest"; the average number was 3 to 5. The designation "oocystic nest-cell" is proposed to describe the cell with such a multiple infection.

Oocysts passed into the cecal lumen usually remain there, or in the large intestine, for a period of 24 to 36 hours, after which they are eliminated with the pellets. If environmental conditions obtained around these pellets are propitious the sporogonic life cycle ensues.

COMPARATIVE INFECTION STUDIES IN COTTONTAILS AND TAME RABBITS

In order to study possible physiological differences between tame rabbits and cottontails, a series of infections was performed, and the resultant symptoms and oocyst elimination are compared.

A total of 54 young tame rabbits, all free from coccidia except for the first ones used, were infected with *E. neoleporis*. With the exceptions of one naturally immune rabbit and a few old does and bucks, all were susceptible.

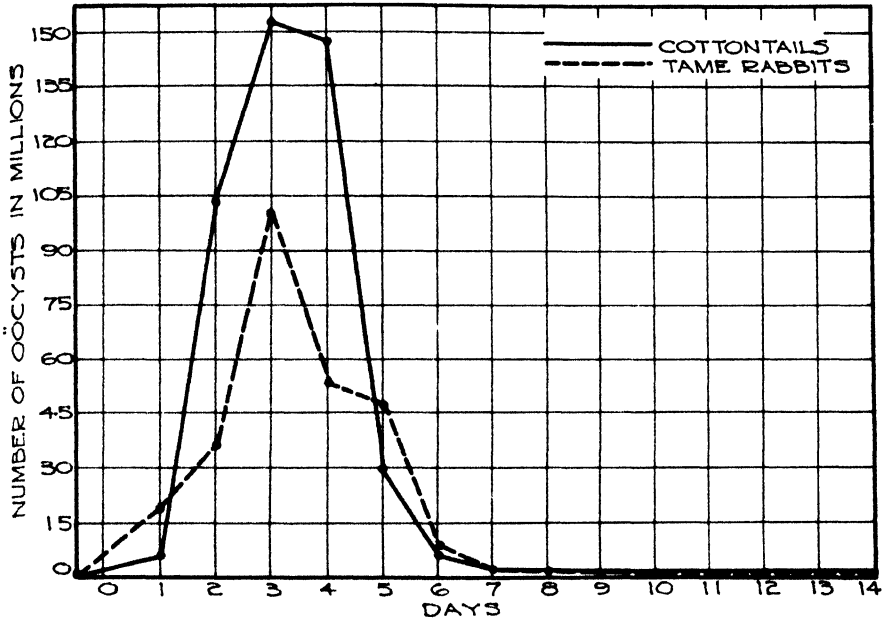
Daily oocyst elimination increased rapidly to a pronounced maximum on the third day, with a moderate decrease after this period. Graph I compares the average numbers of oocysts eliminated daily by five tame rabbits and by three cottontails, each inoculated with 150,000 oocysts.

The prepatent period was found to average 12 days, with a range of from 11 to 14 days. The patent period averaged 10 days, with a range of from 8 to 16 days.

In no case was loss of weight noted, even with dosages as high as 150,000 oocysts. Diarrhea was also absent. The only apparent symptom was partial loss of appetite during the 2 or 3 first days.

Three cottontails, about 34 days old, were injected with 150,000 oocysts each from the same culture as that administered to the tame rabbits. Two of them had a previous infection with *E. environ* (whose oocysts are readily distinguishable from *E. neoleporis*), and the third one was free from coccidia. All were infected. The prepatent period averaged 12 days as in the tame rabbit, with a range of from 12 to 13 days. The patent period averaged 13 days, ranging from 12 to 15.

As might be expected, the natural host showed a much more marked susceptibility to the disease than tame rabbits. Two days before elimination of the first oocysts they became quiet, developed a rough coat, and showed a marked loss of appetite. One of them had diarrhea from 2 days after oocysts were first seen in the pellets. The other two had only softened pellets for a period of 4 days. The most evident symptom was loss of weight, which in one rabbit dropped as much as 50 per cent. All three lost weight from the eighth day after infection up to the third day after oocyst elimination (6 to 8 days). After elimination of



Graph I. Comparative daily oocyst elimination of *E. neoleporis* in infections in tame rabbits and cottontails.

oocysts commenced, the rabbits were fed green alfalfa to simulate natural food.

The numbers of oocysts given off by the cottontail (see graph) were much greater than in the tame rabbits. The elimination curve is roughly similar, but rises abruptly to a high level on the second day of the patent period, reaching a maximum on the third and fourth days, and then dropping sharply to a low level on the fifth day.

It is apparent that despite the large number of oocysts given off daily and the more marked susceptibility of cottontails, there was a general similarity in the development of the infection in both hosts. However, with the dosage given, cottontails lost weight during the infection, while tame rabbits continued to gain. No higher dose than 150,000 oocysts was tried in the cottontail, but comparison of effects of this dosage with lighter ones suggests that still higher doses than this might kill the rabbits.

EFFECTS OF SERIAL PASSAGES IN TAME RABBITS

An infection of *E. neoleporis* was established in a tame rabbit by cross-infection from a cottontail, and then passed through five additional successive generations in tame rabbits.

In each case the infection was positive, with prepatent and patent periods similar to those in the cottontail. No weakening of the parasite was manifest. Sporulation continued normally, and quantitative counts revealed an apparently uniform oocyst elimination throughout the series.

Biometrical studies made during each infection and throughout the passages failed to demonstrate any great degree of size variation. The average dimensions of a hundred oocysts measured daily during the series are given below (average taken from the mode):

Passages						6 (cottontail)
Length.....	38.8	38.0	39.3	39.3	38.5	38.6
Width.....	18.2	18.4	19.8	19.0	19.5	18.8

Oocysts from the fifth passage when brought back to the cottontail produced normal infection with prepatent and patent periods which remained within the average (12 and 11 days, respectively). Measurements taken were also normal.

E. neoleporis is, then, a parasite with the potentiality of gaining a new host in the near future. One case of spontaneous infection was noted in the room where the rabbits were maintained. Results obtained show that no apparent modification, physiological or biometrical, occurred, thus demonstrating the capacity of the tame rabbit to harbor the species. Considering, however, the age-resistance developed by adult rabbits, even after serial passages, the chances are that a considerable number of either natural or artificial reinfections would have to be made before the parasite would adapt and establish itself in old animals. No cases of natural infection were found in tame rabbits examined in Ames. The possibility of its occurrence is, however, not to be excluded.

In the serial passages no variation was noted in the health condition of the hosts. All rabbits gained weight, ate regularly, and signs of diarrhea were not noted.

IMMUNITY

Experiments reported by a number of workers have shown that by administration of sublethal doses of oocysts of various species of *Eimeria*, a partial or total immunity has been developed in the hosts. All results obtained so far have failed to show the development of cross-immunity in the case of species of *Eimeria*. This is the reason why a certain rabbit, immunized to one or two species, may become infected if a third or fourth species is administered or accidentally gains entrance

through the digestive canal. A natural immunity seems to exist, in occasional individuals, at least to *E. neoleporis*, but no experimental work of a genetic nature has been performed to determine whether such immunity is transmitted to the offspring of such individuals.

The author, experimenting with *E. neoleporis* in the tame rabbit, has obtained results which clearly demonstrate the presence of an acquired and lasting immunity against reinfection by this species. Two groups of rabbits, both young and reared free from coccidia were set apart, and different dosages of oocysts were introduced into the stomach. The data obtained and other details are shown in Table 1.

In the first group, composed of 4 rabbits from the same litter, sublethal doses of 80,000 oocysts were administered to two of them; the

TABLE 1
DATA PERTAINING TO IMMUNITY DEVELOPING AFTER INFECTIONS
WITH *Eimeria neoleporis*

	Age in Days	First Infec- tion Date	Dosage in Oocysts	Re- sult	Second Infec- tion Date	Dosage in Oocysts	Re- sult	Third Infec- tion Date	Dosage in Oocysts	Re- sult
Group I										
No. 1...	44	12-21-41	80,000	+	1-14-42	150,000	-	1-30-42	150,000	-
No. 2...	44	12-31-41	80,000	+	1-14-42	150,000	-	1-30-42	150,000	-
No. 3...	44	Control	1-14-42	150,000	+	1-30-42	150,000	-
No. 4...	44	Control	1-14-42	150,000	+	1-30-42	150,000	-
Group II										
No. 5...	22	1-21-42	3,000	+	2-14-42	150,000	+	3-30-42	150,000	-
No. 6...	22	1-21-42	6,000	+	2-14-42	150,000	-	3-30-42	150,000	-
No. 7...	22	1-21-42	12,000	+	2-14-42	150,000	-	3-30-42	150,000	-
No. 8...	22	1-21-42	25,000	+	2-14-42	150,000	-	3-30-42	150,000	-
No. 9...	22	Control	3-30-42	150,000	+

others remaining as controls. Both rabbits were infected positively. When a second dosage of 150,000 oocysts was given to all individuals (controls included), the first two did not show any infection, whereas the controls were infected. A third application of the same amount of oocysts to the entire group was negative, showing that an acquired and lasting immunity was present in the rabbits of group I.

Group II, composed of 4 rabbits and 1 control, from a single brood was infected with doses of 3,000, 6,000, 12,000 and 25,000 oocysts. Only rabbit 5 which received the smallest dose, could be infected a second time when the amount of oocysts were increased to 150,000. Rabbits 6, 7, and 8 were immunized after the first treatment. The control, which was left apart until the third infection trial (of 150,000 oocysts each) was the only rabbit to develop the infection after the third treatment.

Daily oocyst elimination of rabbit 5 after the first infection and rabbit 9 (control) showed that rabbit 5, after being infected with 3,000 oocysts, did acquire a partial immunity to *E. neoleporis*.

Table 2 shows that daily oocyst elimination was much higher in the control than in rabbit 5. In no case during these experiments did

rabbits receiving 80,000 oocysts or more show such a low daily oocyst elimination as in rabbit five, after the first infection with 3,000 oocysts. This proves that light doses of oocysts lead the host to acquire a partial immunity to *E. neoleporis*.

These experiments permit the conclusion that the tame rabbit following a dosage of 6,000 or more oocysts develops an acquired and

TABLE 2
DAILY OOCYST ELIMINATION OF RABBIT 5 (SECOND INFECTION), AND
RABBIT 9 (CONTROL), FROM GROUP II

Days	Rabbit 5	Rabbit 9 (control)
1	less than 100,000	19,000,000
2	1,111,112	21,000,000
3	2,650,000	69,000,000
4	less than 100,000	39,000,000
5	" " "	18,000,000
6	" " "	5,000,000
7	" " "	277,778
8	" " "	150,000
9	" " "	less than 100,000

total immunity to *E. neoleporis*, while lighter doses only lead the host to an acquired and partial immunity.

A natural immunity to *E. neoleporis* was revealed in only one rabbit, (young male, No. 12), which was reared free from coccidia and subjected to three doses of 150,000 oocysts each without positive results; i.e., oocysts were not eliminated. Oocysts of the same culture when inoculated into other rabbits did cause coccidiosis. Due to lack of time the author did not conduct any genetical tests to see if this rabbit could transmit to its litters immunity to *E. neoleporis*. Since *E. neoleporis* is not a species normal to the tame rabbit it seems unwise to attempt an explanation for the immunity in this single rabbit. It is interesting to remember, however, that this was the only case of natural immunity noted in about 68 experimental rabbits.

AGE-RESISTANCE AGAINST *E. NEOLEPORIS*

Five adult tame rabbits, four does and one buck, were given 150,000 oocysts previously tested in young rabbits. Daily examination carried up to the eighteenth day after administration of the sporulated culture failed to reveal the presence of oocysts in the pellets.

This age-resistance may be attributed to the greater similarity of young tame rabbits and cottontails in the physiological reactions, or to a defense mechanism which is not yet fully developed. Infections given to rabbits up to 120 days old were positive. No attempt was undertaken to delineate the exact period when age-resistance begins. In the cottontail there is no evidence of age-resistance to *E. neoleporis* since adult as well as young are readily susceptible to infection, and it occurs in field-captured animals of all ages.

EFFECT OF HOST-COLOR UPON THE INFECTION AND PARASITE

In order to determine whether or not color coat is correlated with the infection or parasite, white and black rabbits were separated in two groups of three. Group I was black; Group II white.

Both groups were represented by young rabbits about 18 days old and free from coccidia. *E. neoleporis* was then given to them in doses of 80,000 oocysts each. The results were shown to be positive for all rabbits, with a prepatent period of 12 to 14 days and a patent period of 8 to 14 days. Table 3 shows the variation on both groups, which remained within the normal range.

TABLE 3
PREPATENT AND PATENT PERIODS

Groups	Group I (Black)			Group II (White)		
Rabbit	1	2	3	1	2	3
Prepatent period	13	14	12	12	13	12
Patent period (days)	14	10	8	11	8	10

Biometrical and quantitative studies have shown also that color is not correlated with number or size of oocysts eliminated. Table 4 shows the amount of oocysts eliminated daily by each individual of the two groups.

The table shows that there is no significance in the numbers above and that host-color has no effect upon the number of oocysts given off

TABLE 4
DAILY OOCYST ELIMINATION OF BOTH GROUPS
(Numbers in Millions)

Groups	Group I (Black)			Group II (White)		
Rabbit No.	1	2	3	1	2	3
Days						
1	3	†	†	†	6	110
2	11	†	25	†	10	610
3	146	56	33	111	42	167
4	22	36	8	35	21	58
5	13	28	†	40	0.1	19
6	2.5	3	†	17	†	3
7	†	†	†	3	†	2.3
8	†	†	*	0.1	*	0.2
9	†	†		—		0.1
10	*	*		*		*
11						
12	†					
13	†					
14	*					

* Last day of the infection.

† Number of oocysts less than 100,000 per 24 hours.

TABLE 5
MEAN LENGTH AND BREADTH OF THE OOCYSTS RECOVERED FROM BLACK AND
WHITE RABBITS (50 FROM EACH RABBIT MEASURED DAILY)

Group.....	Group I (Black)			Group II (White)		
Rabbit.....	1	2	3	1	2	3
Days						
1.....	18.5×40.0	20.0×40.0	20.0×40.0	20.0×40.0	20.0×38.6	20.0×40.0
2.....	18.5×38.6	20.0×40.0	18.5×38.6	20.0×38.6	18.5×40.0	20.0×40.0
3.....	18.5×40.0	20.0×38.6	18.5×40.0	20.0×38.6	18.5×38.6	20.0×40.0
4.....	19.0×38.6	20.0×38.6	18.5×40.0	20.0×38.6	18.5×40.0	18.5×40.0
5.....	20.0×38.6	18.5×38.6	18.5×38.6	20.0×40.0	20.0×38.6	18.5×38.6
6.....	18.5×38.6	20.0×40.0	20.0×40.0	20.0×40.0	18.5×38.6	20.0×38.6
7.....	18.5×38.6	18.5×38.6	18.5×38.6	20.0×38.6	18.5×40.0	18.5×40.0
8.....	18.5×40.0	18.5×38.6	18.5×38.6	18.5×40.0	18.5×38.6	18.5×40.0
9.....	18.5×38.6	18.5×38.6	20.0×40.0	18.5×38.6
10.....	19.0×38.6	20.0×40.0	18.5×40.0	18.5×38.6
11.....	18.5×40.0	20.0×40.0
12.....	20.0×40.0
13.....	18.5×38.6
14.....	19.0×38.6
Mean.....	18.8×39.1	19.5×38.8	18.5×39.0	19.7×39.4	18.8×39.1	19.1×39.4
		18.9×38.9			19.2×39.3	

daily in both groups. It shows also that in spite of the same dosage and same culture, there is an apparent variation in the number of oocysts eliminated per rabbit, which may be due to several factors, such as host-resistance, vitality of the oocysts, etc.

No especial morphological or pathological modifications were apparent in any individual or group. Table 5 shows the mean dimensions of the oocysts per rabbit, per day, as well as per group and throughout the patent period.

Conclusion can thus be made that host-color affects neither the parasite nor the development of the infection in the host.

SUMMARY AND CONCLUSIONS

1. This paper reports studies made by the author with *Eimeria neoleporis*. Experiments were carried on to observe its behavior, life cycle, biometrical or physiological changes, immunity relationships, etc., in the tame rabbit.

2. The endogenous cycle of *E. neoleporis* was studied, and three, probably four generations, of merozoites were found. There is a superimposition in the development of the different generations, and the third generation is apparently bifunctional, giving rise to gametocytes and to asexual merozoites, which continue the asexual phase of the cycle temporarily. Descriptions and figures for the different schizonts, merozoites, macro- and micro-gametocytes, as well as for the intestinal lesions are given.

3. Comparative infection studies were undertaken in tame

rabbits and cottontails. Daily oocyst elimination was found to be much heavier in cottontails than in tame rabbits, when the same amount of oocysts is given to either host. While the infection apparently does not seriously affect tame rabbits, cottontails show a much more marked susceptibility to it, losing as much as 50 per cent of their weight. In some individuals there is an accentuated loss of appetite and diarrhea. The course of the infection was apparently the same in both hosts.

4. Serial passages were executed in tame rabbits. Altogether five successive passages were obtained. Biometrical data yielded during each infection and throughout the series failed to show any size variation. Neither physiological nor pathological modifications were found to exist. Oocysts of the fifth passage when fed again to cottontails caused infection showing normal size and characteristics. *E. neoleporis* is, then, a parasite with the potentiality of gaining a new host in the near future.

5. Immunological tests were carried on with tame rabbits, and the results obtained permit the conclusion that when the dose given is above 6,000 oocysts there is an acquired and total immunity to *E. neoleporis*, while lighter doses lead the host to an acquired and partial immunity. Natural immunity to *E. neoleporis* was found to exist in one young tame rabbit.

6. Experiments on age-resistance to *E. neoleporis* demonstrated that age-resistance exists in adult tame rabbits. Adult cottontails, however, harbored normal and infective oocysts, proving that there is no age-resistance to *E. neoleporis* in this host.

7. The effect of host-color upon the infection and parasite was studied with black and white rabbits. The results showed that host-color bears influence neither upon the infection nor the parasite.

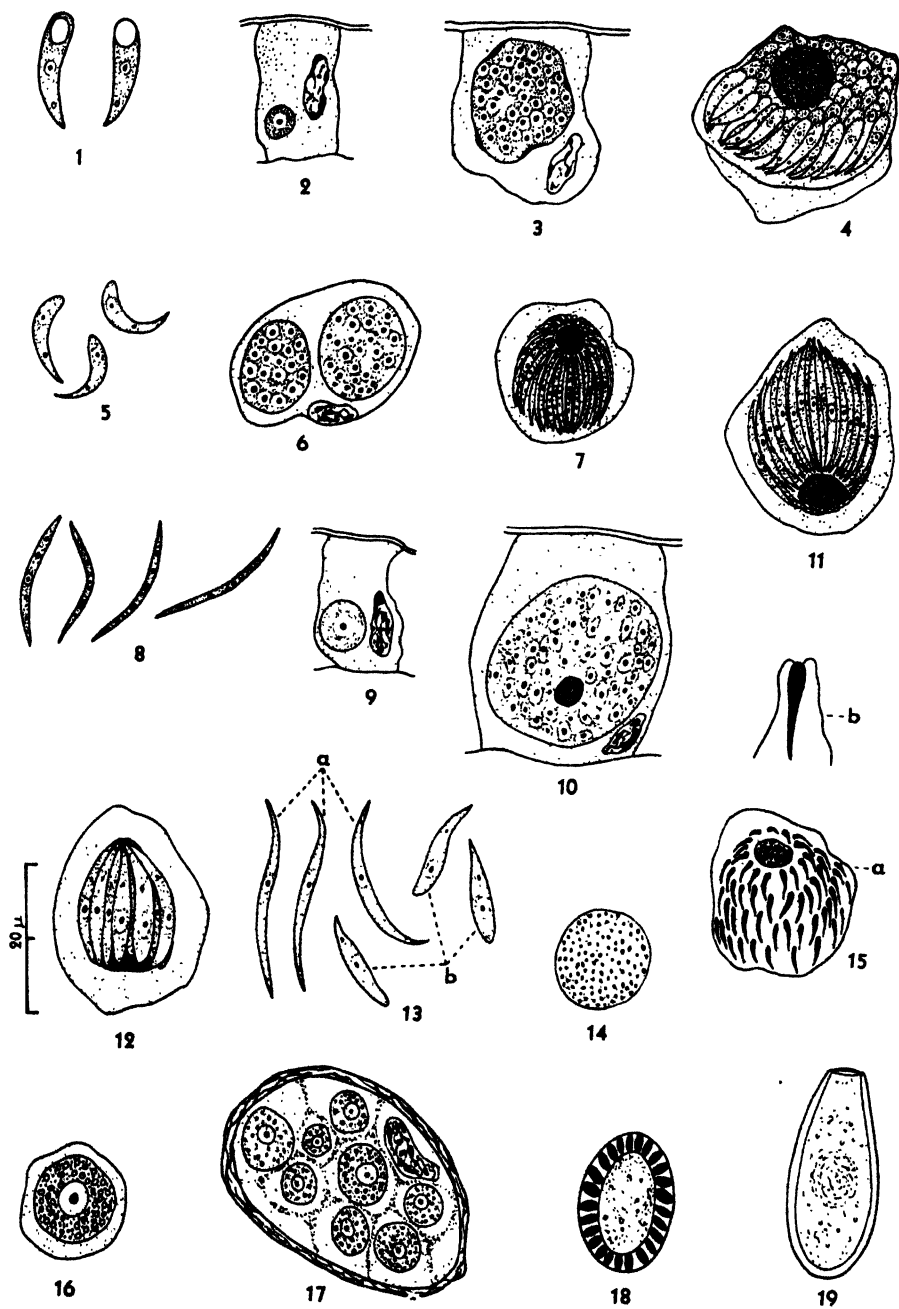
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EXPLANATION OF PLATE I

All figures were drawn under oil immersion with the aid of a camera lucida. The projected scale has the value (in micra) indicated.

- Fig. 1. Sporozoites of *E. neoleporis* recovered from the intestinal lumen.
2. Young schizont of the first generation in host cell.
 3. More advanced schizont of the first generation in host cell.
 4. Adult schizont, with merozoites and residual mass in host cell.
 5. Merozoites of the first generation recovered from the intestinal lumen.
 6. Two grown schizonts of the second generation in host cell.
 7. Adult schizont of the second generation, with merozoites and residual mass in host cell.
 8. Merozoites from the second generation recovered from the intestinal lumen.
 9. Growing schizont of the third generation in host cell.
 10. More advanced schizont of the third generation in host cell.
 11. Adult schizont of the third generation, larger type, with merozoites and residual mass.
 12. Adult schizont of the third generation, smaller type, with merozoites and residual mass.
 13. a. Merozoites of the third generation from the larger type of schizont.
b. *idem*, from the smaller type of schizont.
 14. Growing microgametocyte.
 15. a. adult microgametocytes still within the cell.
b. adult microgametocyte recovered from the intestinal lumen, 5 times the scale on plate.
 16. Growing macrogametocyte.
 17. "Oocystic nest-cell."
 18. Young oocyst showing deposition of plastic granules to form the external wall.
 19. Oocyst still within the tissues.



A NEW HARMOSTES, WITH A PROVISIONAL KEY AND A CHECK LIST TO THE SPECIES (HEMIPTERA: RHOPALIDAE)

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In two earlier studies (Journal of the Washington Academy of Sciences, Vol. 32, pp. 27-32, 1942, and Iowa State College Journal of Science, Vol. 16, pp. 357-362, 1942) notes regarding the recognition features and the synonymy and distribution of some of the lesser-known species of *Harmostes* together with descriptions of several new species were given. This paper characterizes an additional new species and presents a provisional key that has grown out of the earlier studies. Manifestly the key has its shortcomings, but in making use of the texture and punctuation of the hemelytra and the characters of the bucculae, rostrum, and genitalia, as well as the shape of the pronotum and the nature of its lateral margins, it seems to offer something above those that have preceded it.

Harmostes corazonus Distant, *H. brevispinis* Blote, and *H. ochraceus* Blote are unknown to me, and as their original descriptions give insufficient details of the texture and punctuation of the hemelytra it has been necessary to omit them from the key. *H. corazonus* is described as a small species, 5.5 mm., with pale, hyaline membrane and a double series of fuscous spots along the costal margin of the corium and with spots on the nervures. Its third antennal segment is a little longer than the second. The species is probably close to *montivagus* Distant and *marmoratus* Blanchard. Gibson considered it to be a synonym of the latter.

H. brevispinis is 8.5 mm. long. The pronotal margin is white, broader than in *nebulosus*, irregularly and rather coarsely crenulated. The sides of the pronotum, as figured, are sinuate, with the humeral angles broadly rounded. The third antennal segment is distinctly longer than the second, and the membrane is spotted.

H. ochraceus is 8.0 mm. long, yellowish ochraceous, with the head, front of pronotum and scutellum, except apex, darker. Antennal III is distinctly longer than II. The pronotum is said to have the lateral edges finely crenulated and nearly concolorous with the disc, with the front angles situated distinctly behind the front margin. The lateral margin of the pronotum as figured is distinctly concave.

Harmostes splendens, n. sp.

Related to *H. prolixus* Stål, but stouter, with the lateral edge of the pronotum minutely crenulate, and the male clasper much longer and narrower.

Size medium for the genus; body oblong-oval. General color yellowish testaceous, speckled with crimson and with variable brownish to

fuscous markings. Head subequally as broad as long. Spines of antenniferous tubercles short. Bucculae tapering, barely going behind a point opposite front edge of eye. Antennae rather long, segment I surpassing tylus by one-third its own length, II equal to or slightly greater than width of head across eyes; proportions, 15:28:32:19. Rostrum reaching on metasternum; segment I extending much beyond bucculae but not attaining collum. Pronotum coarsely punctate, almost twice as broad as long (58:30), gradually elevated and widened backwards; the sides practically straight, their edges slightly explanate, distinctly reflexed, and minutely crenulate. Hemelytra opaque and coarsely punctate throughout, only a small area along the median furrow smooth. Membrane hyaline, sometimes inconspicuously speckled. Male clasper of the *reflexulus* type, but relatively long and narrow.

LENGTH, 7.3 mm. WIDTH across humeri, 2.5 mm.

HOLOTYPE, male, and ALLOTYPE, female, Santa Cruz, Bolivia, September, 1917; in my collection. PARATYPES, one male taken with types, one female, Bolivia, S. America, three males and six females, Chapada, Brazil; in collections of Carnegie Museum and author.

One female paratype is fresher and more conspicuously marked with brown than the other individuals. The abdomen is marked above with brown, these spots showing through the apex of the corium and disc of membrane. The species is readily placed by its discriminative characters outlined in the following key. It differs from *H. brevispinis* Blote, also from Bolivia and known to me only through the original description and figure, in its almost straight and more finely crenulate lateral edge of the pronotum and the less broadly rounded humeral angles. In collections it has been labeled *subrufus* Distant.

KEY TO SPECIES

1. Hind margin of pronotum deeply and profoundly incised behind humeral angles (see figure by Distant).....*incisuratus* Distant
Hind margin of pronotum entire, not notched within the humeral angles 2
2. Cell Sc (fig. 14) of hemelytra coriaceous, usually strongly punctate, rugulose, or papillose, if semi-transparent then distinctly punctate over major part of surface 3
Cell Sc of hemelytra distinctly hyaline, never uniformly punctate, rugulose, or papillose, but sometimes with a marginal row of punctures 21
3. Hemelytra in large part strongly, coarsely, and thickly punctate, the punctures prominent, usually almost as much so as those of pronotum 4
Hemelytra usually rather distinctly papillose, or rugulose, rarely noticeably punctate and then punctures much less profound than those on pronotum 7
4. Lateral margins of pronotum irregularly but distinctly serrate, at least a few of the teeth discrete and clean cut (fig. 12) 5

- Lateral edge of pronotum smooth or only granulate or obsoletely and minutely crenulate 11
5. Tylus strongly produced, reaching at least to apical fifth of the first antennal segment. Distance from eye to apex of antenniferous tubercle greater than length of eye.....*serratus* (Fabricius)
Tylus less strongly produced, reaching only to about apical third of antennal I. Distance from eye to apex of antenniferous tubercle not greater than length of eye 6
6. Antennal III $\frac{1}{2}$ longer than II. Hind femora shorter. Corium reaching apex of abdomen. From Galapagos Islands
.....*disjunctus* Barber
Antennals II and III usually subequal. Widespread throughout neotropical and tropical America.....*dorsalis* Burm.
7. Lateral edge of pronotum serrate in front, becoming crenulate backwards (fig. 8). First rostral segment reaching front edge of prosternum 8
- Lateral edge of pronotum smooth or only obsoletely granulate or crenulate. First segment of rostrum not or barely going beyond a point opposite hind margin of eyes 9
8. Bucculae of nearly uniform height throughout their length, terminating abruptly opposite front margin of eyes, the extreme hind end free (fig. 3). Pronotum relatively flat, gradually declivent and narrowed anteriorly, the sides nearly straight.....
.....*nebulosus* Stål
Bucculae gradually tapering backwards, going beyond front margin of eyes, the tip not free. Pronotum strongly declivent anteriorly and sinuate laterally, the humeri broadly rounded and somewhat flaring*formosus* Dist.
9. Form broad, the reflexed sides of pronotum wide, the humeri somewhat flaring. Antennal I surpassing tylus by a distance equal to diameter of eye 10
- Smaller and narrower. Pronotum more gradually widened backwards, the sides less strongly explanate. Antennal I shorter and not so incrassate, surpassing tylus by a distance distinctly less than diameter of eye. Membrane immaculate.....*bicolor* Distant
10. Humeri subprominent. Membrane immaculate. Antennal segments more slender*subrufus* Distant
Humeri flaring. Membrane speckled. Antennal segments shorter and stouter*croceus* Gibson
11. Side margin of pronotum consisting of only a raised granulate line, neither explanate nor reflexed. Hemelytra with 3 or 4 cells (R, M, M₁, and Cu) in part hyaline and impunctate.....
.....*angustatus* V.D.
- Sides of pronotum more or less distinctly explanate and usually somewhat reflexed. Hemelytra at most with only one cell (R) in part impunctate 12
12. Antennal I barely exceeding apex of tylus. Hemelytra with a row of dark spots within costal margin. Membrane spotted with brown

-*fraterculus* (Say)
 Antennal I exceeding apex of tylus by $\frac{1}{3}$ to $\frac{1}{2}$ its own length. Costal margin of hemelytra and membrane sometimes spotted but not always so 13
13. Bucculae and first segment of rostrum subequally long, extending to base of head (fig. 2) 14
 First rostral segment $\frac{1}{3}$ to $\frac{1}{2}$ longer than bucculae, these latter not reaching behind a point opposite middle of eyes 16
14. Pronotum rather flat, the sides not or only slightly reflexed, their edges almost straight (fig. 9). Antennae shorter than head, pronotum and scutellum combined. Size less than 5 mm. 15
 Pronotum with lateral margins rather distinctly reflexed. their edges broadly concave. Size large, 6-9 mm.*reflexulus* (Say)
15. Distinctly fusiform. Antennal I, II, and III subequal in length. Membrane immaculate*fusiformis* Harris
 Elongate-oval Antennal III $\frac{1}{3}$ longer than II. Hemelytra and membrane speckled*insitivus* Harris
16. Pronotum regularly and uniformly widened posteriorly, the sides straight or only slightly sinuate. Length of second antennal segment as great as width of head through eyes..... 17
 Pronotum somewhat suddenly widened, the margins broad, their edges noticeably concave. Length of antennal II usually not or barely greater than width of vertex plus one eye 18
17. Form rather elongate, the head longer than wide. Pale reflexed edge of pronotum smooth or practically so. Male clasper short and broad*prolixus* Stål
 Form stouter, the head subequally as wide as long. Reflexed edge of pronotum distinctly, minutely crenulate. Male clasper narrow*splendens* Harris
18. Length 9-10 mm. Antennal II enlarged basally. Male clasper of the *reflexulus* type (figs. 6 and 7)*raphimerus* Spinola
 Small species, the length not over 6.5 mm. Second antennal segment stoutest at apex. Male clasper of the *procerus* type (fig. 5) 19
19. Reflexed side margin of pronotum broad, as wide as an eye. Width of pronotum fully $2\frac{1}{2}$ times median length.....*gemellus* Harris
 Side margin of pronotum not nearly as wide as an eye. Width of pronotum barely 2 times its median length 20
20. Membrane immaculate*marmoratus* (Blanchard)
 Membrane speckled*montivagus* Distant
21. Lateral margins of pronotum distinctly explanate and reflexed. Bucculae embracing not much more than basal half of first rostral segment 22
 Side margin of pronotum consisting of only a raised, granulate line whose width is less than diameter of second antennal segment. Bucculae embracing first rostral segment for its entire length*corizoides* Jensen-Haarup

22. Lateral edge of pronotum strongly concave, the humeral angles flaring and projecting outward so that distance across them is distinctly greater than width across base of hemelytra (fig. 11)*confinis* Harris
Sides of pronotum almost straight or only slightly concave, the humeral angles neither raised nor flaring and not or barely projecting beyond sides of hemelytra 23
23. Form rather elongate, the head slightly longer than broad, the width of pronotum distinctly less than twice its median length. Second antennal segment usually somewhat enlarged at base. Membrane usually with more or less distinct dark streaks..... 24
Form proportionally broader, the head not or barely as long as broad; the pronotum twice as broad as long. Second antennal segment not enlarged basally. Membrane immaculate 25
24. Pronotum relatively flat, only feebly impressed each side of median raised line. Hind margin of genital segment of male broadly, convexly produced at the middle (fig. 5)*procerus* Berg
Pronotum more strongly arched, the impressions on each side of median raised line broader and more profound. Hind margin of male genital capsule truncate, not produced (fig. 4).....*imitabilis* Harris
25. Bucculae gradually tapering backwards 26
Bucculae only slightly tapering, terminating abruptly, the hind end almost truncate (fig. 1)*petulans* Harris
26. Length of second antennal segment subequally as great as width of head across eyes. Pronotal margin narrow, rather sharply reflexed*apicatus* Stål
Length of antennal II not greater than diameter of vertex plus one eye. Pronotal margin broader, only slightly reflexed*minor* (Spinola)

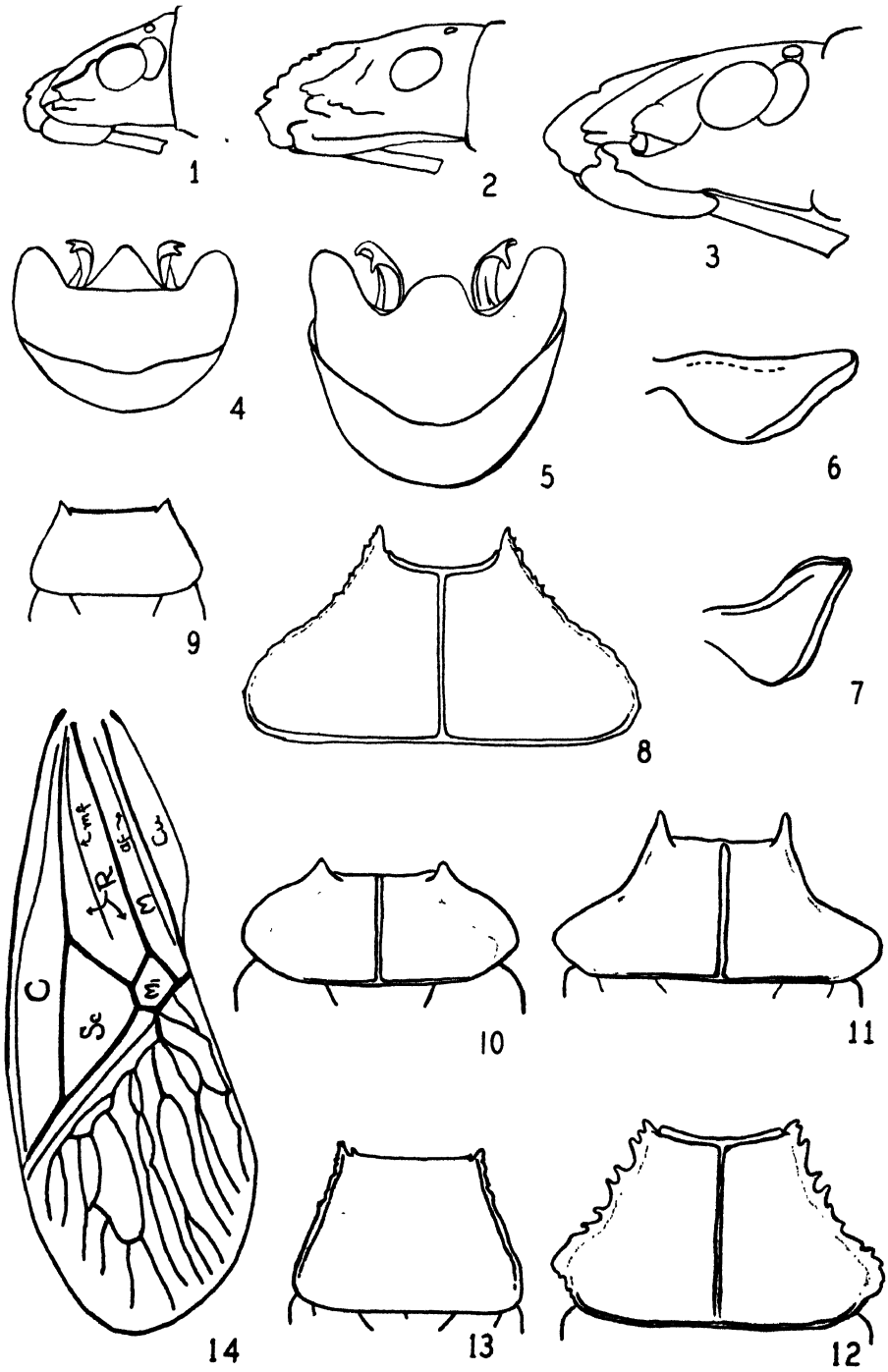
LIST OF SPECIES WITH THEIR RANGE

- affinis* Dallas, 1852 (= *dorsalis* Burmeister; new synonymy)
- angustatus* Van Duzee, 1918.....Calif., Ariz., N. Mex., Tex.
- apicatus* Stål, 1860Argentina, Paraguay, Bolivia, Brazil
- bicolor* Distant, 1881Mexico, S. W. United States
- brevispinis* Blote, 1934Bolivia
- bruesi* Bergroth, 1913 (= *reflexulus* Say)
- chilensis* Dallas, 1852 (= *minor* Spinola)
- confinis* Harris, 1942Chile
- corizoides* Jensen-Haarup, 1924....Argentina
- corazonus* Distant, 1893Chile
- costalis* Herrich-Schaeffer, 1853.... (= *reflexulus* Say)
- croceus* Gibson, 1917Calif., Ore., Tex.
- disjunctus* Barber, 1925Galapagos Islands

<i>dorsalis</i> Burmeister, 1835	Mexico, Tex., Fla., West Indies, Central and South America
<i>formosus</i> Distant, 1881	Mexico
<i>fraterculus</i> (Say), 1832	New Jersey south to Fla. and west to Calif.
<i>fusiformis</i> Harris, 1942	Peru
<i>gemellus</i> Harris, 1942	Argentina, Peru
<i>gracilis</i> Reed, 1900	(belongs to <i>Xenogenus</i> Berg)
<i>gravidator</i> Fabricius, 1794	(= <i>serratus</i> Fabricius)
<i>imitabilis</i> Harris, 1942	Argentina
<i>incisuratus</i> Distant, 1881	Columbia
<i>insitivus</i> Harris, 1942	Chile
<i>marmoratus</i> (Blanchard), 1852....	Chile
<i>minor</i> (Spinola), 1852	Chile
<i>montivagus</i> Distant, 1893	Chile
<i>nebulosus</i> Stål, 1862.....	Mexico, Guatemala
<i>ochraceus</i> Blote, 1934	Venezuela
<i>perpunctatus</i> Dallas, 1852	(= <i>serratus</i> Fabricius)
<i>petulans</i> Harris, 1942	Bolivia, Peru, Argentina
<i>procerus</i> Berg, 1879	Argentina, Uruguay
<i>prolixus</i> Stål, 1860	Brazil, Paraguay, Bolivia, Argentina
<i>propinquus</i> Distant, 1881	(belongs to <i>Aufeius</i> Stål)
<i>raphimerus</i> (Spinola), 1852	Chile
<i>reflexulus</i> (Say), 1832	throughout United States
<i>serratus</i> (Fabricius), 1794	Tropical America
<i>signoreti</i> Reed, 1900	(= <i>raphimerus</i> Spinola)
<i>splendens</i> Harris, n. sp.	Bolivia, Brazil
<i>subrufus</i> Distant, 1881	Guatemala; Tex.
<i>virescens</i> Dallas, 1852	(= <i>reflexulus</i> Say)

EXPLANATION OF FIGURES

- Fig. 1. Head of *H. petulans* Harris.
 2. Head of *H. reflexulus* (Say).
 3. Head of *H. nebulosus* Stål.
 4. Male genital segments of *H. imitabilis* Harris.
 5. Male genital segments of *H. procerus* Berg.
 6. Male paramere of *H. reflexulus* (Say).
 7. Male paramere of *H. dorsalis* Burm.
 8. Pronotum of *H. nebulosus* Stål.
 9. Pronotum of *H. insitivus* Harris.
 10. Pronotum of *H. gemellus* Harris.
 11. Pronotum of *H. confinis* Harris.
 12. Pronotum of *H. serratus* (Fabr.).
 13. Pronotum of *H. procerus* Berg.
 14. Typical venation of *Harmostes* hemelytron.



A PRELIMINARY LIST OF HEMIPTERA OF IDAHO

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Idaho is of especial interest to the collector and student of insect distribution, because of its varied topography and great range of latitude. This preliminary list of the *Hemiptera* of the state is the outgrowth of much general collecting by various workers over a period of years and by the senior author during the months June to August, inclusive, 1938. Comparable lists are available for British Columbia by Downes¹ and for Utah by Knowlton and Harmston².

The British Columbia list records 196 species, exclusive of the miridae and corixidae, and the Utah list records 134 species exclusive of these two families. In comparison the present study discloses the known occurrence in Idaho of 233 forms. Unquestionably the list will be considerably expanded as more intensive collecting is done in the state.

It is desirable in a study of this type to give seasonal distribution as well as geographical distribution and other pertinent data concerning the different species. Because of the limitations of publication space, however, it has been necessary to exclude much of this data. The localities are cited alphabetically, and only the earliest and latest dates of seasonal capture are given. Some forms are recorded without specific names or are questionably referred to a given species. This has seemed desirable because they represent possible unnamed forms and are in the hands of specialists for study or are represented by too few specimens for accurate identification. The miridae and corixidae are yet to be identified.

Through the courtesy of Mr. J. R. Douglass it has been possible to include records of certain species in the collections of the Twin Falls, Idaho, Laboratory of the Bureau of Entomology and Plant Quarantine. These are indicated with the asterisk.

Family SCUTELLERIDAE

Homaemus aeneifrons (Say). Kendrick, Moscow Mt.; Aug. 13–20.

Homaemus bijugis Uhl. Carey, Grangeville, Kendrick, Moscow Mt.,
Parma, Shoshone, Troy; June 19–Aug. 26.

Eurygaster alternatus (Say). Bovill, Buhl, Burley, Clarkia, Coeur d'Alene,
Genesee, Grangeville, Hollister, Kootenai, Lowman, McCall, Mos-

¹Downes, W., A preliminary list of the Heteroptera and Homoptera of British Columbia. Proc. Ent. Soc. of British Columbia, No. 23, 1927, pp. 3–22.

²Knowlton, G. F., and F. C. Harmston, Utah insects, Hemiptera, Utah Agr. Exp. Sta., Mimeo. Ser. 200 (Tech.), part 6, 1940.

- cow, Potlatch, Rigby, Salmon, Santa, Troy, Twin Falls; May 30—Aug. 27.
- Eurygaster shoshone* Kirk. Juliaetta, Kendrick, Lewiston, Moscow Mt.; April 4—Aug. 13.
- Vanduzeeina* sp. Moscow Mt.; July 20.

Family CYDNIDAE

- Pangaeus discrepans* Uhl. (Recorded by Van Duzee, 1904).
- Aethus obliquus* (Uhl.).* Burley, Hazelton, Hollister, Hubbs Butte, Jerome; May 25—Sept. 12.
- Aethus testudinatus* Uhl.* Burley, Twin Falls; June 27—July 1, in wind vane trap.
- Galgupha nitiduloides* (Wolf). (Recorded from the state by McAtee and Malloch).
- Amnestus spinifrons* (Say). Hollister, Lewiston; May 8—Nov. 3.
- Cydnoides renormatus* (Uhl.).* Hollister; Sept. 18, in wind vane trap.
- Geotomus parvulus* Sign.* Wendell; Aug. 17, in wind vane trap.
- Allocoris extensa* (Uhl.). Ashton, Bruneau, Grangeville, Kendrick, Lenore, Moscow Mt., Santa, Troy, Weippe; May 10—Aug. 27.
- Allocoris pulicaria* (Germar). Moscow, Santa, Troy; March 7—Aug. 27.
- Allocoris incognita* McAtee and Malloch. Kootenai, Moscow Mt.; July 8—Aug. 20.
- Allocoris nigra* (Dallas). Clarkia, Moscow, Troy; June 28—Aug. 21.
- Allocoris virilis* McAtee and Malloch. Bruneau, Camas Prairie, Craters of Moon, Mountain Home, Parma, Weiser; June 15—July 21.

Family PENTATOMIDAE

- Brochymena quadripustulata* (Fabr.). Boise, Emmett, Moscow, Parma; April 12—Oct. 11.
- Brochymena affinis* Van Duzee. Moscow; March 11—Oct. 15.
- Brochymena* sp. Regina, June 23. A unique female, apparently near *B. sulcata* Van Duzee.
- Peribalus limbolarius* Stål. Buhl, Castleford, Emmett, Hollister, Kendrick, Lewiston, Moscow, Sandpoint, Santa, Shoshone; May 4—Oct. 21.
- Peribalus abbreviatus* Uhl. Craters of Moon, Genesee, Kendrick, Moscow Mt., Payette; May 23—Oct. 9.
- Peribalus tristis* Van Duzee. Moscow Mt., Santa; July 10—Aug. 27.
- Trichopepla grossa* Van Duzee. Kootenai, Lenore, Moscow Mt.; May 10—July 20.
- Trichopepla aurora* Van Duzee. Juliaetta, Moscow Mt.; May 10—July 20.
- Trichopepla californica* Van Duzee. Grangemont, Moscow Mt.; May 13—Aug. 26.
- Chlorochroa uhleri* Stål. Bruneau, Burley, Clarkia, Grangeville, Hagerman, Jerome, Kendrick, Montpelier, Moscow Mt., Nezperce, Regina, Rexburg, Rigby, Rupert, Shoshone, Twin Falls; June 14—Sept. 10. Occasionally very numerous on wheat in southeastern Idaho.
- Chlorochroa sayi* Stål. Twin Falls; June 17—July 25.

- Chlorochroa ligata* (Say). Craters of Moon, Kootenai, Lenore, Moscow; May 19—Oct. 29.
- Carpocoris remotus* Horvath. Burley, Castleford, Grangeville, Hollister, Kendrick, Moscow, Oakley, Potlatch, Rexburg; May 2—Oct. 19.
- Euschistus conspersus* Uhl. Bovill, Juliaetta, Kendrick, Kootenai, Lewiston, Moscow, Potlatch, Troy; May 28—Aug. 24.
- Euschistus variolarius* (P. B.). Genesee, Juliaetta, Kendrick, Parma, Preston, Rigby; May 16—Aug. 13.
- Euschistus inflatus* Van Duzee. Boise, Burley, Magic Reservoir (Shoshone), Moscow Mt., Twin Falls; June 11—Sept. 23.
- Coenus delius* (Say). Kendrick; Aug. 13.
- Neottiglossa undata* (Say). Lewiston, Moscow Mt., Potlatch, Santa, Troy; May 10—Aug. 27.
- Neottiglossa tumidifrons* Downes. Craters of Moon, Kendrick, Lenore, Moscow; May 28—Aug. 26.
- Cosmopepla bimaculata* (Thomas). Salmon; July 8.
- Cosmopepla conspicillaris* (Dallas). Mesa, Orofino, Santa; July 17—Aug. 27.
- Cosmopepla uhleri* Montandon. Buhl; Aug. 1.
- Eysarcoris intergressus* (Uhl.). Kendrick, Moscow Mt.; July 13—20.
- Prionosoma podopoides* Uhl. Bruneau, Regina; July 23.
- Thyanta custator* (Fabr.). Buhl, Bruneau, Dayton, Hollister, Kendrick, Lenore, Lewiston, McCall, Moscow, Riggins; March 1—Oct. 14.
- Thyanta rugulosus* (Say). Bruneau, Regina, Twin Falls; June 18—23.
- Thyanta punctiventris* Van Duzee.* Glenns Ferry, Tuttle; April 6—Aug. 27.
- Thyanta brevis* Van Duzee. Buhl, Aug. 1.
- Banasa sordida* (Uhl.). Moscow Mt.; July 20.
- Banasa dimidiata* (Say). Kootenai, Moscow Mt.; May 6—Oct. 20.
- Meadorus lateralis* (Say). Recorded in the literature from Idaho. Specimens are at hand from Oregon and Washington.
- Elasmotherus cruciatus* (Say). Santa; July 4—Aug. 21.
- Perillus bioculatus* (Fabr.). Bruneau, Lewiston, Moscow, Parma, Salmon, St. Anthony; Feb. 20—Aug. 26.
- Perillus bioculatus* var. *clauda* (Say). Jerome, Moscow Mt., Parma, Salmon; July 10—Aug. 26.
- Perillus exaptus* (Say). Santa; Aug. 27.
- Apateticus crocatus* (Uhl.). Moscow Mt., Potlatch; Aug. 8—26.
- Apateticus bracteatus* (Fitch). Record from the literature, Van Duzee, 1917.
- Podisus modestus* (Dallas). Moscow Mt.; July 26.
- Podisus sereiventris* Uhl. Recorded from Idaho by several authors.
- Podisus placidus* (Uhl.). Rigby; June 28.
- Podisus pallens* Stål. Lewiston, Moscow Mt.; April 4—Aug. 24.

Family COREIDAE

- Leptoglossus occidentalis* Heideman. Lenore, Lewiston, Moscow Mt., Parma, Santa, Shoup; Apr. 5—Dec. 13.

- Chelinidea vittiger* Uhl. Craters of Moon, Rexburg, St. Anthony; June 15—Sept. 16.
- Anasa tristis* DeGeer. Caldwell, Emmett, Fruitland, King Hill, Parma, Payette, Preston; Jan. 15—Aug. 29.
- Coriomieris humilis* (Uhl.). Athol, Kootenai, Salmon, Shoshone; June 19—July 9.
- Ceraleptus pacificus* Barber. Lewiston, Moscow; May 6—July 10.

Family RHOPALIDAE

- Harmostes reflexulus* (Say). Genesee, Hollister, Kendrick, Kootenai, Lewiston, Moscow, Regina, Rupert, Tuttle, Twin Falls, Weippe; Apr. 22—Aug. 16.
- Aufeius impressicollis* Stål. Buhl, Craters of Moon, Eureka, Fruitland, Hollister, Lewiston, Parma, Riggins, Twin Falls; June 15—Oct. 27.
- Liorhyssus hyalinus* (Fabr.). Craters of Moon, Hagerman, Hailey, Hollister, Montpelier, Parma, Twin Falls; June 23—Sept. 17.
- Stictopleurus punctiventris* (Dallas). Grangemont, Kendrick, Lenore, Lewiston, McCall, Montpelier, Moscow, Potlatch, Troy, Weippe; Apr. 16—Oct. 14.
- Stictopleurus plutonius* (Baker)? Bruneau, Buhl, Burley, Craters of Moon, Dubois, Jerome, Ketchum, Regina, Twin Falls; June 14—Aug. 26.
- Arhyssus scutatus* (Stål). Hubbs Butte; June 15.
- Arhyssus indentatus* (Hambleton). Moscow, Parma; May 2—July 10.
- Arhyssus validus* (Uhl.). Avon, McCall, Moscow, Potlatch; June 14—Aug. 24.
- Arhyssus lateralis* (Say). Caldwell, Fruitland, Lenore; May 19—July 20.
- Arhyssus lateralis roseus* (Baker). Bruneau, Glens Ferry, Rigby; June 23—Sept. 5.
- Arhyssus barberi* Harris. Hagerman, Kendrick, Lewiston, Moscow Mt., Troy, Tuttle, Twin Falls; Apr. 22—Sept. 3.
- Arhyssus crassus* Harris. Grangeville, Lenore, Lewiston, Moscow, Parma, Troy; Apr. 28—Aug. 21.
- Arhyssus brevipilis* Harris. Kendrick, Kootenai, Moscow Mt.; July 8—20.
- Arhyssus tuberculatus* (Hambleton). Kendrick, Moscow Mt., Troy; July 13—Oct. 21.
- Leptocoris trivittatus* (Say). Boise, Moscow, Nampa, Potlatch; Apr. 10—Dec. 1.

Family CORISCIDAE

- Megalotomus quinquespinosus* (Say). Coeur d'Alene, Moscow Mt., Potlatch; July 20—Aug. 26.
- Coriscus pluto* (Uhl.). Moscow, Potlatch; June 10—Aug. 24.
- Coriscus conspersus* (Montd.). Ashton, Buhl, Clarkia, Fruitland, Hollister; Aug. 4—20.
- Coriscus conspersus infuscatus* Fracker. Moscow Mt.; Aug. 26.
- Tollius curtulus* (Stål.) Kendrick; Aug. 13.

Tollius setosus (Van Duzee). Bovill, Craters of Moon, Kendrick, Moscow Mt.; June 26—Aug. 13.

Family ARADIDAE

Aradus furnissi Usinger. Moscow Mt., Aug. 20.

Aradus funestus Bergr. Moscow; Apr. 16—Dec. 1.

Aradus lugubris Fallen. Moscow Mt., Sandpoint; Apr. 2—Oct. 3.

Aradus lugubris nigricornis Reuter. Weippe; July 5.

Aradus cinnamomeus antennalis Parshley. Moscow Mt., Santa, Worley; May 1—Aug. 27.

Aradus vadosus Van Duzee. Moscow; Mar. 12, under bark of poplar.

Aradus medioximus Parshley. Moscow Mt., Potlatch; June 6—Aug. 24.

Aradus blaisdelli Van Duzee. Craig Mts., Moscow Mt.; June 6—Aug. 26.

Aradus proboscideus Walker. Moscow Mt., Troy; July 16—Aug. 20.

Aradus taylori Van Duzee. McCall; Aug. 10.

Aradus debilis Uhl. Moscow Mt.; Aug. 20.

Aradus parvicornis Parshley. Moscow Mt., Aug. 26.

Aradus abbas Bergr. Bonanza, Rexburg; June 22—Aug. 21. (Literature records).

Aradus inoletus Van Duzee. Worley; May 14.

Aradus orbiculatus Van Duzee. Moscow Mt.; Oct. 29.

Aneurus simplex Uhl. Lake Chatcolet, Moscow, Pierce; May 22—July 17.

Mezira pacifica Usinger. Moscow Mt.; Apr. 5—Aug. 20.

Family NEIDIDAE

Neides muticus (Say). Grangeville, Hollister, Kootenai, Lewiston, Moscow, Santa, Troy; Apr. 17—Oct. 11.

Jalysus wickhami Van Duzee, Donnelly, Hagerman, Hollister, Kimama, Kootenai, Moscow, Troy, Tuttle; May 19—Oct. 19.

Acanthophysa idaho Harris. Grangeville; June 20.

Acanthophysa echinata Uhl. Craters of Moon, Regina; June 23—28.

Family LYGAEIDAE

Lygaeus kalmii Stål. Buhl, Cascade, Clarkia, Fruitland, Glens Ferry, Hailey, Hollister, Montpelier, Moscow Mt.; Apr. 6—Sept. 24.

Lygaeus admirabilis Uhl. Kootenai, Moscow Mt.; July 8—20. These specimens are very small and brachypterous and are questionably referred to this species.

Lygaeus bicrucis Say. Genesee, Lenore, Moscow; Apr. 16—Aug. 26.

Lygaeus pyrrhopterus Stål. Buhl; Aug. 1.

Lygaeus lateralis Dallas. Magic Reservoir, Lincoln Co.; June 25.

Ortholomus scolopax (Say). Craters of Moon, Kendrick, Kootenai, Lenore, Moscow Mt., Potlatch, Troy, Tuttle; May 19—Aug. 24.

Ortholomus nevadensis Baker. Buhl; June 24.

Nysius ericae (Schill.). Carey, Eureka, Juliaetta, Kootenai, Moscow, Regina, Rexburg, Rigby, Salmon; May 28—Sept. 26.

Nysius minutus Uhl.* Burley, Hagerman, Hansen, Hollister; May 31—Oct. 15.

- Nysius strigosus* Uhl. Craters of Moon, Hazelton, Hollister, Kootenai, Moscow, Potlatch, Rupert, Twin Falls; July 2—Aug. 7.
- Nysius thymi* (Wolff).* Buhl, Castleford; Sept. 18—Oct. 17.
- Nysius* sp. Kootenai, Moscow, Regina, Santa; Mar. 3—Aug. 27.
- Ischnorrhynchus resedae* (Panzer). Buhl, Moscow Mt., Santa; July 10—Aug. 27.
- Ischnorrhynchus franciscanus* (Stål). Craters of Moon, Kendrick, Kootenai, Moscow, Potlatch, Troy; June 26—Oct. 26.
- Cymus luridus* Stål. Bovill, Caldwell, Grangemont, Harvard, Moscow, Potlatch, Santa, St. Anthony; May 17—Aug. 27.
- Arphnus coriacipennis* (Stål). Moscow, Moscow Mt.; Mar. 10—July 23.
- Geocoris bullatus* (Say). Bovill, Buhl, Eureka, Kendrick, Moscow, Parma, Rigby, Roseworth, Shoshone, Twin Falls; Mar. 10—Aug. 17.
- Geocoris decoratus* Uhl. Eureka, Fruitland, Kendrick, Kootenai, Lewiston, Moscow Mt., Potlatch, Riggins; June 20—July 23.
- Geocoris atricolor* (Montd.). Fruitland, Moscow Mt., Tuttle, Wendell; Feb. 5—Sept. 16.
- Geocoris uliginosus* (Say). Bovill, Cascade, Kendrick, Kootenai, Moscow Mt., Troy; July 8—Aug. 26.
- Heterogaster behrensi* (Uhl.). Bellevue, Hailey, Twin Falls; June 14—July 30.
- Crophius bohemani* (Stål). Kendrick, Lewiston, Moscow; May 8—Aug. 13.
- Crophius ramosus* Barber. Burley, Hansen, Hollister, Hubbs Butte, Jerome; May 29—June 16.
- Crophius scabrosus* Uhl.* Hollister, Twin Falls; Oct. 8—16.
- Sphaerobius insignis* (Uhl.). Hollister, Kootenai, Moscow; June 24—Aug. 4.
- Ligyrocoris diffusus* (Uhl.). Bovill, Genesee, Kootenai, Moscow Mt., Troy; July 2—17.
- Ligyrocoris latimarginatus* Barber. Kendrick, Moscow Mt.; July 10—Aug. 20.
- Ligyrocoris sylvestris* (Linn.). Bovill, Clarkia, Kendrick, Moscow Mt., Troy; July 13—Aug. 21.
- Sisamnes clavigera* (Uhl.). Kendrick, Moscow; Mar. 10—Aug. 13.
- Neosuris castanea* (Barber). Kendrick; Aug. 13.
- Peritrechus fraternus* Uhl. Buhl, Moscow, Regina, Wendell; June 23—Nov. 6.
- Peritrechus tristis* Van Duzee. Moscow, Troy; Feb. 24—Nov. 15.
- Peritrechus saskatchewanensis* Barber. Jerome, Moscow, Rupert, Troy; July 30—Aug. 21.
- Cligenes* sp.? Kendrick; Aug. 13.
- Sphragistus nebulosus* (Fallen). Juliaetta, Moscow, Potlatch, Santa, Twin Falls; Mar. 10—Aug. 26.
- Malezonotus angustatus* (Van Duzee). Moscow Mt., Parma, Santa; Feb. 24—Nov. 14.

- Malezonotus sodalicus* (Uhl.). Lewiston, Moscow, Moscow Mt.; Mar. 10—Aug. 16.
Emblethis vicarius Horvath. Burley, Hagerman, Hubbs Butte, Kendrick, Lewiston, Moscow, Potlatch; Mar. 10—Oct. 6.
Eremocoris melanotus Walley. Moscow; Apr. 2.
Eremocoris semicinctus Van Duzee. Moscow; Oct. 14.
Eremocoris obscurus Van Duzee. Moscow Mt., Santa, Troy; July 10—Aug. 27.
Eremocoris canadensis Walley. Moscow Mt.; Mar. 11.
Scolopostethus pacificus Barber. Moscow, Moscow Mt., Potlatch; Mar. 10—Nov. 16.
Scolopostethus thomsoni Reuter. Moscow, Troy; Mar. 10—July 16.
Scolopostethus tropicus Distant (?) Moscow; Mar. 10—May 10.
Cryphula apicatus (Distant). Kendrick; Aug. 13.

Family PIESMIDAE

- Piesma cinerea* (Say). Jerome, Moscow, Potlatch, Riggins; Mar. 10—Sept. 4.

Family TINGITIDAE

- Corythucha immaculata* O. & D. Hailey, Moscow Mt., Santa; Apr. 21—July 4.
Corythucha distincta O. & D. Moscow; May 15.
Corythucha padi Drake. Moscow, Potlatch; Aug. 8—24.
Corythucha obliqua O. & D. Burley, Moscow, Tuttle; June 1—Sept. 9.
Corythucha salicata Gibson. Council, Jerome, Lewiston, Moscow Mt., Potlatch, Santa, Twin Falls; May 8—Aug. 27.
Corythucha morrilli O. & D. Amsterdam, Murtaugh; July 23—Aug. 19.
Corythucha marmorata Uhl. Kootenai; July 8.
Gargaphia solani Heideman. Lewiston; May 5.
Physatocheila opacula Uhl. Regina; June 23.
Physatocheila variegata Parshley. Lewiston, Rigby, St. Anthony; May 30—June 28.
Hesperotingis occidentalis Drake. Kootenai, McCall; July 8—31.
Teleonemia nigrina Champion. Potlatch, Santa; June 20—Aug. 27.
Monanthia labeculata Uhl.* Hollister; Oct. 5.

Family PHYMATIDAE

- Phymata borica* Evans. Pocatello. (Evans' record).
Phymata metcalfi Evans. Hansen, Wickahoney; July 29—Aug. 3.
Phymata pennsylvanica coloradensis Melin. Arrow, Kendrick, Moscow, Riggins; Aug. 6—Oct. 2.

Family REDUVIIDAE

- Empicoris pilosus* (Fieber). Moscow Mt.; Aug. 20.
Empicoris culiciformis (De Gerr). Moscow; April.

- Empicoris* sp. (?). Moscow Mt.; July 10.
Metapterus banksi (Baker). Moscow Mt.; Oct. 15.
Reduvius personatus (Linn.). Fruitland, Kendrick, Moscow, Parma;
 June 29—July 25.
Zelus socius Uhl. Buhl, Craters of Moon, Sun Valley, Twin Falls; June
 25—Aug. 27.
Rhynocoris ventralis (Say). Kendrick, Moscow, Troy, Weippe; Apr. 28
 —July 28.
Pselliopus spinicollis Champion. Kendrick, Lewiston, Moscow Mt.; Apr.
 21—July 13.
Fitchia spinulosa Stål. Kendrick, Moscow, Parma; Apr. 3—Aug. 6.
Sinea diadema (Fabr.). Buhl, Eureka, Kendrick, Lewiston, Moscow Mt.,
 Parma, Regina, Troy; May 27—Aug. 26.
Sinea confusa Caudell.* Tuttle; Sept. 19.

Family NABIDAE

- Pagasa fusca* (Stein). Kendrick, Moscow, Moscow Mt., Rigby, Riggins;
 June 20—Sept. 5.
Nabis nigrovittatus Sahlberg. McCall; Aug. 10.
Nabis heidemanni (Reuter). Kendrick, Moscow Mt.; July 20—Aug. 26.
Nabis subcoleoptratus (Kirby). Clarkia, Moscow, Potlatch, Santa; July
 5—Aug. 27.
Nabis alternatus Parshley. Bruneau, Buhl, Clarkia, Grangeville, Ken-
 drick, Kootenai, Orofino, Parma, Potlatch, Rexburg, Rupert, Sand-
 point, Sun Valley, Troy; June 20—Aug. 24.
Nabis roseipennis Reuter. Moscow Mt., Potlatch, Santa, Troy; Aug.
 15—27.
Nabis ferus (Linn.). McCall, Moscow Mt., Potlatch, Rupert, Troy; July
 20—Aug. 24.
Nabis vanduzeei Kirkaldy. Grangeville, Lenore, Montpelier; May 19—
 June 27.
Nabis inscriptus Kirby. Literature records. Ranges from Colorado into
 Alberta, Canada.
Nabis rufusculus Reuter. Moscow Mt.; Sept. 20—Oct. 14.

Family CIMICIDAE

- Cimex pilosellus* (Horvath). Boise; Nov. 10.
Cimex lectularius Linn. Fairfield, Kendrick, Moscow, Parma; July 24—
 Aug. 27.

Family ANTHOCORIDAE

- Anthocoris antevolens* White. Bovill, Buhl, Cascade, Hazelton, Moscow,
 Potlatch, Rigby, Salmon, Santa, St. Anthony; Mar. 10—Oct. 10.
Anthocoris melanocerus Reuter. Bovill, Hailey, Moscow Mt., Rigby,
 Sandpoint, Twin Falls; Apr. 14—July 17.
Anthocoris whitei Reuter. McCall, Moscow Mt.; July 10—Aug. 26.
Tetraphleps latipennis Van Duzee. Moscow Mt.; July 10—Aug. 26.

- Tetraphleps* sp. Moscow Mt.; Aug. 26.
Acompocoris sp. McCall; July 31.
Orius tristicolor (White). Buhl, Challis, Eureka, Gooding, McCall, Moscow, Potlatch, Regina, Rigby, Santa; June 18—Aug. 27.
Lyctocoris campestris Fabr. Moscow Mt.; Aug. 26.
Lyctocoris sp. Moscow Mt.; Aug. 26.
Dufouriellus ater (Dufour). Lewiston, Moscow Mt.; Feb. 22—Aug. 26.
Xylocoris galactinus Fieber.* Castleford, Hansen, Jerome, Tuttle; Aug. 5—Sept. 15.
Xylocoris cursitans (Fallen). Moscow, Moscow Mt., Santa, Troy; July 16—Oct. 8.
Xylocoris umbrinus Van Duzee. Hansen, Lewiston, Moscow Mt., Potlatch, Troy, Twin Falls; Apr. 30—Sept. 30.
Xylocoris sp. Parma; June 22.
Xylocoris californicus Reuter. Hagerman, Twin Falls; July 14—Sept. 19.
Scoloposcelis flavicornis Van Duzee. Moscow Mt.; Aug. 15.

Family GERRIDAE

- Gerris notabilis* Drake and Hottes. Bovill, Lake Chatcolet, Parma, Potlatch, Troy; June 18—Sept. 2.
Gerris remigis Say. Moscow, Twin Falls; Apr. 3—Oct. 13.
Gerris nyctalis Drake and Hottes. Caldwell, Kendrick, Moscow, Troy; July 13—Oct. 20.
Gerris incurvatus Drake and Hottes. Caldwell, Moscow Mt., Potlatch, Troy; July 9—Sept. 12.
Gerris pingreensis Drake and Hottes. Listed by Drake and Hottes as occurring in Idaho.
Gerris incognitus Drake and Hottes. Bovill, Lake Chatcolet, McCall; July 17—Aug. 10.
Gerris buenoi Kirkaldy. Lake Chatcolet, Potlatch; July 17—Sept. 2.
Metrobates trux (Torre Bueno). Caldwell, Kendrick, Santa; July 6—Aug. 27.

Family VELIIDAE

- Microvelia borealis* Torre-Bueno. Potlatch; July 23.
Rhagovelia excellentis Drake and Harris. Sweetwater, Webb; July 26—Sept. 3.

Family MESOVELIIDAE

- Mesovelia mulsanti* White. Lake Chatcolet; Sept. 2.

Family SALDIDAE

- Salda obscura* Provancher. Bovill; July 17.
Saldula interstitialis (Say). Bovill, Bruce Meadows, Cascade, Coeur d'Alene, Elk River, Hazelton, Kootenai, Lake Chatcolet, Magic Reservoir, Moscow Mt., Potlatch, Regina; June 15—Aug. 26.
Saldula reperta Uhl. Lake Chatcolet, Santa; July 17—Aug. 27.

Saldula xanthocheila (Fieber). Deer Flat, Hazelton, Riggins; June 20—Aug. 7.

Saldula comatula Parshley. Coeur d'Alene, Fruitland, Hazelton, Kootenai, Moscow Mt., Potlatch, Rupert, Troy; July 8—Aug. 24.

Saldula explanata (Uhl.). Bovill, Moscow Mt.; July 10—Aug. 26.

Micracantha pusilla Van Duzee. Moscow Mt., Twin Falls; July 11—Aug. 26.

Family NOTONECTIDAE

Notonecta kirbyi Hungerford. Bliss, Bovill, Burley, Council, Moscow, Moscow Mt., Troy; June 25—Oct. 11.

Notonecta unifasciata Guerin. Moscow; May 15.

Buenoa sp. Riggins; Aug. 10.

Family NEPIDAE

Ranatra fusca P. B. Potlatch, Troy; Aug. 24—Sept. 12.

Family BELOSTOMATIDAE

Lethocerus americanus Leidy. Moscow; July 8.

Lethocerus uhleri Montd. Moscow; July 20.

Family GELOSTOCORIDAE

Gelostocoris californiensis Melin. Moscow, Pollock, Starkey, Troy; July 2—Aug. 26.

A LIST OF FLEAS (SIPHONAPTERA) COLLECTED AT TAMA, IOWA¹

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During the summer and fall of 1941 the authors collected a number of fleas (*Siphonaptera*) on the Tama Indian Reservation, Tama, Iowa. A total of sixteen flea species from eighteen different species of host animals were recorded, including fifteen unrecorded hosts. The fleas were obtained while the authors were engaged in tick studies on the Reservation. As many small mammals were "live trapped" or shot during the course of the studies, information on the occurrence and seasonal abundance of several species of fleas was secured.

The fleas were cleared and mounted according to the technique employed by Fox (1940). Unless otherwise stated all collections were made at Tama, Iowa, during the summer of 1941. The records are summarized by giving the total number of specimens of a species taken per month, followed in parentheses by the number of males and females.

FAMILY PULICIDAE

Cediopsylla simplex Baker

On *Sylvilagus floridanus mearnsii*: April, 657 (♂♂ 293, ♀♀ 362) specimens on 13 host animals, averaging 50.5 per animal; May, 526 (♂♂ 248, ♀♀ 278) specimens on 10 hosts, average 52.6 per animal; June, 344 (♂♂ 164, ♀♀ 180) fleas on 5 hosts, average 68.8; July, 18 (♂♂ 9, ♀♀ 9) specimens on 4 animals, average 4.5 per animal; Aug., 121 (♂♂ 41, ♀♀ 80) on 5 hosts, average 24.2; Sept., 61 (♂♂ 26, ♀♀ 35) on 5 hosts, average 12.2; Oct., no fleas on two animals examined; Nov., 4 (♂♂ 3, ♀♀ 1) on 6 hosts, average .66; Dec., 94 (♂♂ 39, ♀♀ 55) on 6 hosts, average 15.6. On *Canis familiaris*: April, 10 (♂♂ 7, ♀♀ 3) specimens; May, 2 (♂♂ 1, ♀♀ 1); July, 1 ♀. No effort was made to tabulate the number of dogs examined for fleas. The carnivorous habit of the dog probably accounts for the rabbit fleas taken on that animal. This is by far the commonest species of flea from the Mearns cottontail in the area studied. The 56 cottontails examined averaged 32.6 fleas of this species per animal. Of the total number of cottontails examined 78.6 per cent were found to be infested, and 98.1 per cent of all fleas taken from them were *C. simplex*. The female fleas usually outnumbered the males, 825 specimens being males, 1000 females.

¹The authors are indebted to Dr. G. M. Kohls, of the Rocky Mountain Spotted Fever Laboratory, Hamilton, Montana, for verifying the identification of most of the species. Appreciation is also extended to Dr. Walter L. Bierring and Dr. Carl Jordan of the Iowa Health Department for financial assistance and to Dr. C. J. Drake of Iowa State College for his interest and encouragement in the investigations.

Hoplopyllus affinis Baker

On *Sylvilagus floridanus mearnsii* Allen: Nov. 24, 1 female flea at Ames, Iowa; June 18, 1942, 20 (♂ ♂ 16, ♀ ♀ 4) specimens from nest, Ames, Ia. On *Peromyscus leucopus noveboracensis* Fischer: Jan. 31, 1942, 3 ♀ ♀ specimens at Ames, Ia., from 17 mice examined. This flea, which is usually considered a common parasite of various species of rabbits and hares, was not found in the Tama area. It, however, is common in some of the western states (Kohls, 1940).

Ctenocephalides canis Curtis

On *Canis familiaris*: April, 1 ♀; May, 17 (♂ ♂ 5, ♀ ♀ 12); June, 42 (♂ 1, ♀ ♀ 41); July 15 (♂ ♂ 4, ♀ ♀ 11); July, 1942, 111 (♂ ♂ 44, ♀ ♀ 67). On *Felis domestica*: June 12, 5 ♀ ♀; Sept. 8, 3 ♀ ♀. The dog flea was very common on many dogs on the Reservation, although little effort was made to collect them. Only the above mentioned collections were made and recorded from the dog and the house cat from the large number of these animals examined during the course of the tick studies (1,132 dogs, 22 house cats).

Ctenocephalides felis Bouche

On *Canis familiaris*: July 31, 1942, 5 (♂ ♂ 3, ♀ ♀ 2). This was the only record of the cat flea obtained on the Reservation.

FAMILY DOLICHOPSYLLIDAE

Ctenophthalmus pseudagyrtes Baker

On *Peromyscus leucopus noveboracensis* Fischer: May, 3 (♂ 1, ♀ ♀ 2); June, 5 (♂ ♂ 3, ♀ ♀ 2); Aug., 2 (♂ 1, ♀ 1); Sept., 2 ♂; Oct., 2 ♂; Dec., 1 ♂. On *Reithrodontomys megalotis dychei* Allen: Dec. 9, 1 ♀. On *Microtus pennsylvanicus pennsylvanicus* Ord.: Aug. 22, 1 ♀. On *Mustela longicauda spadix* Bangs: April 28, 1 ♂. On *Spilogale interrupta* Rafinesque: April 22, 1 ♀. On *Scalopus aquaticus machrinoides* Jackson: Oct. 24, 82 (♂ ♂ 43, ♀ ♀ 39); April 5, 1942, 4 (♂ ♂ 2, ♀ ♀ 2), at Ames, Ia. On *Blarina brevicauda brevicauda* Say: May, 12 (♂ ♂ 5, ♀ ♀ 7); June, 2 ♂ ♂; July, 5 (♂ ♂ 2, ♀ ♀ 3); Sept., 6 (♂ ♂ 5, ♀ 1); Dec., 4 (♂ 1, ♀ ♀ 3). On *Geomys bursarius* Shaw: June 21, 1 ♂ specimen. The harvest mouse, *Reithrodontomys megalotis dychei*, and the spotted skunk, *Spilogale interrupta*, are new host records. The flea was quite common on the short-tailed shrew, mole, and the Northern white-footed mouse, 82 specimens being collected on one mole. A total of 29 fleas were taken from 19 shrews. Seventy-eight and nine-tenths per cent of the shrews were infested with fleas, 52.7 per cent of them of this species. As this flea is recorded in the literature from many animals, it evidently parasitizes almost any mammal with which it comes into contact.

Conorhinopsylla stanfordi Stewart

On *Glaucomys volans volans* Linnaeus: Oct. 24, 1 ♂. This one specimen was obtained from the 19 fox squirrels and 5 flying squirrels examined. It is a parasite of squirrels.

Opisocrostitis bruneri Baker

On *Citellus tridecemlineatus tridecemlineatus* Mitchell: April, 28 (♂ ♂ 13, ♀ ♀ 15); May, 2 (♂ 1, ♀ 1); June, 5 (♂ ♂ 4, ♀ 1); July, 4 (♂ ♂ 2, ♀ ♀ 2); Aug., 35 (♂ ♂ 13, ♀ ♀ 22). On *Sciurus niger rufiventer* Geoffroy: Aug. 22, 1 ♂. On *Canis familiaris*: May 19, 1 ♂. On *Peromyscus leucopus noveboracensis* Fischer: May 6, 1 ♀. On *Citellus franklini* Sabine: Aug. 28, 1 ♀. The records of *Opisocrostitis bruneri* from the fox squirrel, dog, and the northern white-footed mouse are new host records. Normally this species is a parasite of ground squirrels.

Oropsylla arctomys Baker

On *Marmota monax monax* Linnaeus: May, 81 (♂ ♂ 32, ♀ ♀ 49); June, 27 (♂ ♂ 15, ♀ ♀ 12); Aug., 5 (♂ ♂ 3, ♀ ♀ 2). On *Peromyscus leucopus noveboracensis* Fischer: May 6, 1 ♀. This is a new host record, apparently accidental, since only one specimen was obtained from the large number of white-footed mice examined. The woodchuck is the normal host, and 113 specimens were taken from eleven animals.

Odontopsyllus multispinosus Baker

On *Sylvilagus floridanus mearnsii* Allen: April, 13 (♂ ♂ 8, ♀ ♀ 5); May, 13 (♂ ♂ 6, ♀ ♀ 7); June, 1 ♀; Dec., 5 (♂ ♂ 3, ♀ ♀ 2). On *Canis familiaris*: May 26, 1 ♀; May 29, 2 (♂ 1, ♀ 1). The usual host of this flea is the cottontail rabbit. Its occurrence on the dog may be attributed to the carnivorous habit of this animal.

Orchopeas wickhami Baker

On *Sciurus niger rufiventer* Geoffroy: May, 52 (♂ ♂ 21, ♀ ♀ 31); June, 8 (♂ ♂ 2, ♀ ♀ 6); Aug., 6 (♂ 1, ♀ ♀ 5); Sept., 32 (♂ ♂ 6, ♀ ♀ 26). On *Glaucomys volans volans* Linnaeus: Sept., 1 ♀; Oct., 10 (♂ ♂ 6, ♀ ♀ 4). This flea is common on the fox squirrel since fourteen of the twenty-four squirrels examined were infested, averaging 4.1 fleas per squirrel. Eleven fleas were also obtained from 5 flying squirrels. Tree squirrels are considered its normal hosts.

Orchopeas leucopus Baker

On *Peromyscus leucopus noveboracensis* Fischer: April, 36 (♂ ♂ 11, ♀ ♀ 25); May, 165 (♂ ♂ 68, ♀ ♀ 97); June, 54 (♂ ♂ 21, ♀ ♀ 33); July, 49 (♂ ♂ 19, ♀ ♀ 30); Aug., 49 (♂ ♂ 20, ♀ ♀ 29); Sept., 117 (♂ ♂ 44, ♀ ♀ 73); Oct., 60 (♂ ♂ 19, ♀ ♀ 41); Nov., 16 (♂ ♂ 4, ♀ ♀ 12); Dec., 28 (♂ ♂ 8, ♀ ♀ 20); Jan. (1942), 2 (♂ 1, ♀ 1) at Ames, Ia.; Feb. (1942), 5 (♂ 2, ♀ ♀ 3), at Ames; Mar. (1942), 14 (♂ ♂ 2, ♀ ♀ 12), at Ames. On *Microtus pennsylvanicus pennsylvanicus* Ord.: Mar., 5 (♂ 1, ♀ ♀ 4); May, 8 (♂ ♂ 3, ♀ ♀ 5); Aug., 2 ♀ ♀. On *Reithrodontomys megalotis dychei* Allen: Sept. 11, 5 (♂ 1, ♀ ♀ 4); Oct. 14, 1 ♀; Nov. 14, 1 ♀. On *Sylvilagus floridanus mearnsii* Allen: Dec. 13, 1 ♂. On *Glaucomys volans volans* Linnaeus: Oct. 24, 1 ♂. On *Canis familiaris*: May 19, 1 ♀. On *Rattus norvegicus* Erxleben: June 20, 1 ♀; June 23, 1 ♀. On *Marmota monax*

monax Linnaeus: June 4, 1 ♀. On *Blarina brevicauda brevicauda* Say: May 25, 1 ♂. And on *Didelphis virginiana virginiana* Kerr.: Aug. 12, 1 ♂. Although principally a parasite of the white-footed mouse, this species lives on many species of small mammals. Six new host records, namely, the harvest mouse, cottontail rabbit, flying squirrel, dog, rat, and woodchuck, are listed. It is one of the most abundant species of fleas on the Tama Indian Reservation, probably due to the fact that the population of the white-footed mouse was high. Specimens were taken from the white-footed mouse every month of the year. The numbers collected each month do not necessarily show the seasonal abundance trend. A total of 2,656 specimens of the white-footed mouse were trapped during the year, and only a portion of the fleas were removed each month. Since the authors were principally interested in the ticks they did not attempt to collect all the fleas from the mice other than to get a representative collection each month. The collections show that *Orchopeas leucopus* is active on the mouse every month of the year, although it was not as abundant during the fall and winter months as during the summer. Of the 845 fleas removed from the white-footed mouse, 70.4 per cent or 595 were of this species. The collections for January, February, and March were made at Ames, Ia., in 1942, since the field studies at Tama, Ia., were terminated in December, 1941.

Megabothris wagneri Baker²

On *Peromyscus leucopus noveboracensis* Fischer: May 12, 1 ♀; May 14, 1 ♂; May 17, 1 ♂; May 18, 1 ♂; May 24, 2 ♀ ♀. This is evidently the first record of this flea on the northern white-footed mouse. It had only previously been recorded from the flying squirrel in Iowa.

Nosopsyllus fasciatus Bosc.

On *Rattus norvegicus* Erxleben: June 23, 4 (♂ 1, ♀ ♀ 3). This flea which occurs on the rat is almost worldwide in distribution. It is thought to be a possible agent in the dissemination of bubonic plague and endemic typhus in some localities.

FAMILY HYSTRICHOPSYLLIDAE

Nearctopsylla genalis Baker

On *Scalopus aquaticus machrinoides* Jackson: Oct. 24, 4 ♂ ♂; April 5, (1942), 2 ♀ ♀, Ames, Iowa. On *Blarina brevicauda brevicauda* Say: Dec. 2, 1 ♀; Dec. 5, 1 ♂. The flea is known from small mammals in the eastern part of the United States. It seems to prefer shrews and moles as hosts. Fox (1940) does not give host data for the collection record from Iowa. The above records therefore verify the flea's occurrence on the shrew and mole in this state. Hubbard (1940) seems to have had some doubt as to the actual occurrence of this flea on the common garden

² There is some question as to the validity of Fox's action in placing *wagneri* in the genus *Megabothris*.

mole, *Scalopus aquaticus*. Only two moles were examined, and specimens were taken from each.

Doratopsylla curvata Rothschild

On *Blarina brevicauda brevicauda* Say: May 25, 16 (♂♂ 9, ♀♀ 7); June 7, 1♂; July 17, 1♂; Sept. 10, 2 (♂ 1, ♀ 1). This flea seems to be quite specific to the short-tailed shrew, since it was the only animal from which it was taken in the Tama area. Thirty-six and three-tenths per cent of all fleas taken from the shrew were of this species.

Epitedia wenmanni Rothschild

On *Microtus pennsylvanicus pennsylvanicus* Ord.: Dec. 8, 2 (♂ 1, ♀ 1). On *Reithrodontomys megalotis dychei* Allen: Nov. 6, 1♀; Nov. 23, 1♀. On *Peromyscus leucopus noveboracensis* Fischer: April, 1♀; May, 6 (♂ 1, ♀♀ 5); June, 2 (♂ 1, ♀ 1); July, 1♀; Sept., 15 (♂♂ 4, ♀♀ 11); Oct., 31 (♂♂ 8, ♀♀ 23); Nov., 101 (♂♂ 37, ♀♀ 64); Dec., 60 (♂♂ 20, ♀♀ 40); Feb. (1942), 6 (♂♂ 2, ♀♀ 4); Mar. (1942), 1♀. On *Blarina brevicauda brevicauda* Say: Dec. 5, 1♀; Dec. 8, 2♂♂. Although principally a parasite of the white-footed mouse, the flea was taken from three other species of animals. The meadow and the harvest mouse are two previously unrecorded hosts. Specimens were taken from the white-footed mouse throughout the year with the exception of the months of August and January. Activity was greatest during the fall and early winter months. During the months of November and December this species exceeded *Orchopeas leucopus* in number recorded on the northern white-footed mouse. Twenty-six and five-tenths per cent of the total number of fleas collected from the white-footed mouse were of the former species.

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From 19 mice: *Epitedia wenmanni* Rothschild, 2; *Orchopeas leucopus* Baker, 15; *Ctenophthalmus pseudagyrtes* Baker, 1.
14. Northern white-footed mouse, *Peromyscus leucopus noveboracensis* Fischer
Epitedia wenmanni Rothschild, 224; *Orchopeas leucopus* Baker, 595; *Hoplopsyllus affinis* Baker, 3; *Opisocrostitis bruneri* Baker, 1; *Oropsylla arctomys* Baker, 1; *Megabothris wagneri* Baker, 6; *Ctenophthalmus pseudagyrtes* Baker, 15. 2,656 mice were trapped but all fleas were not saved.
15. Prairie harvest mouse, *Reithrodontomys megalotis dychei* Allen
From 92 mice examined; *Ctenophthalmus pseudagyrtes* Baker, 1; *Orchopeas leucopus* Baker, 7; *Epitedia wenmanni* Rothschild, 2.
16. Barn rat, *Rattus norvegicus* Erxl.
Three animals examined: *Nosopsyllus fasciatus* Bosc., 4; *Orchopeas leucopus* Baker, 2.
17. Western fox squirrel, *Sciurus niger rufiventer* Geof.
Orchopeas wickhami Baker, 98; *Opisocrostitis bruneri* Baker, 1, from 24 squirrels examined.
18. Mearns cottontail, *Sylvilagus floridanus mearnsii* Allen
From 56 individuals examined: *Cediopsylla simplex* Baker, 1,825; *Odontopsyllus multispinosus* Baker, 32; *Hoplopsyllus affinis* Baker, 1; and *Orchopeas leucopus* Baker, 1.

SUMMARY

A total of 3,298 fleas representing sixteen species were collected and identified by the authors from material collected mainly on the Tama Indian Reservation at Tama, Ia. These were taken from eighteen different species of host animals. Fifteen previously unrecorded host records are

given in the list: two for *Ctenophthalmus pseudagyrtes*, three for *Opisocrostis bruneri*, one for *Oropsylla arctomys*, six for *Orchopeas leucopus*, one for *Megabothris wagneri*, and two for *Epitedia wenmanni*. Some of the records are perhaps accidental occurrences, especially those from the carnivores.

Observations were also made on the seasonal abundance of some of the species of fleas. *Cediopsylla simplex* was taken from the Mearns cottontail every month from April through December. The fact that no rabbits were obtained on the Reservation during the first three months of the year probably accounts for the lack of records during these months. Specimens of *Orchopeas leucopus* were taken from the Northern white-footed mouse every month during the year. However, the fleas of various species were found to be most abundant during the summer months. *Cediopsylla simplex*, the common rabbit flea, and *Orchopeas leucopus*, the common flea of the white-footed mouse, were the most abundant flea species on the area studied.

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LIFE CYCLE OF *CAPILLARIA CAUDINFLATA*, A NEMATODE PARASITE OF THE COMMON FOWL¹

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INTRODUCTION

Studies reported on capillaria worms in poultry during recent years have emphasized the economic importance of this group of nematodes. From various reports, including those of Perroncito and Tomiolo (1899), Allen and Gross (1926), and Hung (1926), it is evident that the presence of *Capillaria annulata* in the crop of chickens, turkeys, and other gallinaceous birds leads to malnutrition, emaciation, and severe anemia accompanied by a desquamation of the infected tissues. Severe inflammation and sloughing of the intestinal mucosa have frequently been reported in pigeons (Eber, 1917; Schlegel, 1918; Wehr, 1939) and chickens (Levine, 1938) infected with the pigeon capillariid, *C. columbae*. Furthermore, Olson and Levine (1939) reported a leucocytosis and slight anemia accompanying *C. columbae* infection in chickens.

Early in 1938, the writer found that chickens examined at Charles City, Iowa, were infected with *Capillaria caudinflata*,³ a species hitherto unreported from the United States. Since heavy infestation with these worms was commonly associated with severe enteritis, diarrhea, and emaciation, further information concerning this species was sought.

A survey of the literature revealed that nothing was known of the method whereby *C. caudinflata* is transmitted from one fowl to another or of the environmental factors which might influence such transmission. Consequently, an investigation regarding its geographical distribution, its life history, and other related factors was begun in November, 1938.

REVIEW OF LITERATURE

The literature concerning the nomenclature of *Capillaria caudinflata* is very confusing. Rudolphi (1819) described the genus *Trichosoma*, listing it as "Genus II. *Trichosoma* (*Capillaria*, Zederi)." Although the synonymy of *Trichosoma* R. with *Capillaria* Zeder, 1800, was thus admitted by Rudolphi, the priority of Zeder's genus was not generally recognized until about a century later.

¹ A condensation of the original thesis submitted to the Graduate Faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, on file at the Iowa State College Library, Ames, Iowa. Doctoral Thesis No. 692A.

² It is with sincere appreciation that the writer acknowledges the helpful advice of Dr. E. R. Becker in outlining and conducting the various experiments.

Thanks are also extended to Dr. J. E. Salsbury, who has generously provided research facilities for conducting this investigation at Dr. Salsbury's Laboratories, Charles City, Iowa.

³ Credit is hereby given Dr. E. E. Wehr for identification of these worms.

Under the genus *Trichosoma*, Rudolphi (1819) described the species *T. longicolle* which he obtained from certain galliform birds. The morphological characteristics which he gave, however, offer no clue by which *T. longicolle* may be distinguished from closely related species. Probably the most valuable clue given is the hosts from which the worms were obtained, that is, "Hab. In intestinis praesertim crassis *Phasiani galli* et *colchici*; in coecis *Tetraonis Urogalli*, *Perdici* et *Tetricis*." But the apparent lack of host-specificity among capillarids as a group and the fact that several species may occur in the digestive tract of a single host at any one time make even this information of little value to the taxonomist.

In the year 1858, Molin described a new species of nematode from *Perdix coturnix* which he called *Calodium caudinflatum*. A translation of Molin's description of this species follows:

Calodium caudinflatum

Body capillary, the male on both sides (probably means throughout the length of the body), the female tapering anteriorly; the epidermis of the caudal extremity of the male inflated into a large transparent ellipsoidal bubble; the spicule sheath tubular, transversely striated, spicule very long and thread-like extending from the terminal bursa opposite a point cut in, from below, in the caudal end; caudal extremity of the female (with) rounded off end; opening of the anus subterminal, lateral; opening of the vulval bursa projecting out in the anterior part of the body, opening bilobed, inner lip the longer. Length of the male 17 mm.; female 25 mm.

Habitat: *Perdix coturnix*; in small intestine, Junio, Patavi (Molin).

In 1861 Molin again published the same description, with one exception, together with figures of the caudal portion of the male and the region of the genital aperture of the female. In his first paper he described the inner lip of the vulval bursa as the longer ("labio interno longiori") but in 1861 he described it as "labio externo longiori." Since his figures of this region of the female show the outer lip the longer, his earlier description was evidently erroneous.

A study of Molin's description together with his figures of *Calodium caudinflatum* leave little doubt that he was describing the same species of nematode which the writer has found in chickens of the United States. On the other hand, there is reasonable doubt regarding the identity of the nematodes which Rudolphi called *Trichosoma longicolle*. In fact, it appears that Shipley (1909) was right when he suggested that Rudolphi may have been dealing with more than one species of "trichosomes." Thus far none of the capillarids of the chicken is known to occur in both the cecum and small intestine, whereas Rudolphi obtained part of his specimens from the intestine and part from the cecum. Therefore, the writer is of the opinion that the name *T. longicolle* should be abandoned in favor of the species name given by Molin. Since the genus *Calodium* is now considered a synonym of the genus *Capillaria* (see Travassos, 1915, and Baylis, 1931), the name *Capillaria caudinflata* (Molin, 1858) is regarded as the correct name for the capillarid whose life cycle is described in this paper.

The literature on *Capillaria caudinflata* (= *Trichosoma longicolle*

=*Capillaria longicollis*) has been written, for the most part, by European helminthologists. Only five papers by North American investigators have thus far been found where this species is mentioned, viz., Stiles and Hassall (1894), Hassall (1896), Beach and Freeborn (1936), Morehouse (1939), and Allen and Wehr (1942).

No specific identification of *C. caudinflata* from birds of the United States had been reported in the literature until 1939 when the writer reported this species from the intestine of chickens from Iowa, Minnesota, Ohio, Illinois, Wisconsin, Pennsylvania, Missouri, Kansas, Indiana, and Michigan.

Among the early European papers concerning *C. caudinflata* are those of Rudolphi (1819); Dujardin (1845); Molin (1858, 1861); Eberth (1863); Diesing (1851, 1861a, 1861b); and Kowalewski (1894, 1901).

Shipley (1909), an English author, identified capillarids found in the red grouse *Lagopus scoticus* as *Trichosoma longicolle* Rud. and listed *Calodium caudinflatum* Molin, *Trichosoma gallinum* Kowal., and *Trichosoma caudinflatum* Kowal. as synonyms. Shipley's paper was the earliest comprehensive treatise on this species of nematode. His description and figures make it almost certain that he was dealing with the same nematode found by the writer in chickens of the United States although it must be remembered that he obtained his specimens from an entirely different species of gallinaceous birds. On the other hand, host-specificity as a general rule seems to be of little taxonomic value when dealing with the capillarids. Experiments reported in the present paper show it to be lacking in *Capillaria caudinflata*.

Morgan (1932), another English author, described nematodes from the English domestic fowl which he identified as *Capillaria longicollis* (Rud., 1819) Travassos, 1915. Clapham (1935), a third English author, reported *Capillaria longicollis* from 44 out of 380 birds examined. She stated that it is a common parasite of the small intestine of many gallinaceous birds including the pheasant, *Phasianus colchicus*. Others who have found this nematode parasite are Huus (1931) who reported *Capillaria longicollis* from the Norwegian ptarmigan, *Lagopus lagopus*, and Mönnig (1933) who found this species in the small intestine of chickens of Utrecht. On the contrary, Reis *et al.* (1936) stated that *C. longicollis* is not found at São Paulo, Brazil.

The lower portion of the digestive tract of the chicken (i.e., the portion posterior to the gizzard) is parasitized by several species of *Capillaria* other than *C. caudinflata*. Rudolphi (1819) described the pigeon capillarid now known as *Capillaria columbae*. This nematode, frequently found in the small intestine of chickens, enjoys a wide geographical distribution as a parasite of the latter host.

Von Linstow (1873) described another capillarid from the intestine of the chicken which he called *Trichosoma collare*. However, Morgan (1932) suggested that the worms described by von Linstow may have been the same as those described by Railliet (1893) under the name *Trichosoma retusum*. If such is the case, then the name of von Linstow's

species becomes a synonym of *Capillaria retusa* (Railliet, 1893). Both species have been described as having annular constrictions in the cuticula near the anterior end, and both are said to have spiny spicule sheaths. On the other hand, *Capillaria collaris* (v. Linstow, 1873) was described as having two lateral bacillary bands whereas *Capillaria retusa* has a third broad ventral band. The fact that von Linstow found his specimens in the intestine of the chicken while Railliet found his in the cecum constitutes another point of difference.

Capillaria gallinum (Kowal., 1894) was originally described from the intestine of the chicken but has since been made a synonym of *C. caudinflata*. Kowalewski (1894) described another species from the cecum of the chicken which he called *Trichosoma dubium*. This name is now considered a synonym of *Capillaria retusa*.

More recently (1934) a species of *Capillaria* was described by Teixeira de Freitas and Lins de Almeida from the small intestine of chickens at Rio de Janeiro, Brazil. The chief distinguishing characteristic of this new species, which they called *Capillaria bursata*, is the morphology of the caudal extremity of the male. It was described as having caudal alae and a membranous copulatory bursa supported by four papillae, two of which are curved ventrally terminating in the margin of the bursa, the other being latero-dorsal in position.

From the foregoing discussion, we may conclude that four and possibly five distinct species of *Capillaria* are known to occur in the lower digestive tract of *Gallus domesticus* L.; namely *Capillaria caudinflata* (Molin, 1858), *C. columbae* (Rudolphi, 1819), *C. bursata* Teixeira de Freitas and Lins de Almeida, 1936, and possibly *C. collaris* (v. Linstow, 1873), all of which occur in the small intestine; the only species thus far found in the cecum is *C. retusa* (Railliet, 1893).

Reports of the successful experimental transmission of worms belonging to the genus *Capillaria* to avian hosts are few indeed. Cram (1931) was able to transmit *Capillaria contorta*, a parasite of the upper digestive tract of numerous birds, to a quail and a domesticated duck by feeding embryonated eggs obtained from a European pheasant. She was, however, unable to transmit this species to chickens, but later (1936) reported its successful experimental transmission to turkeys.

In 1931, Cram also reported the transmission of another capillarid, *C. retusa*, which occurs in the lower digestive tract of various birds. She stated that she was able to produce an infestation in a chicken and a quail by feeding a 41-day-old culture of embryonated eggs collected from a hungarian partridge. On the other hand, Levine (1938) stated that Cram (1937—personal communication to Dr. Levine), after a re-study of her specimens, is convinced that she was working with *C. columbae* and not *C. retusa*. Therefore, it appears that the experimental transmission of *C. retusa* still remained undemonstrated.

Capillaria columbae is perhaps the easiest species to transmit since the period of embryonation is short and no intermediate host is required. Levine (1938) confirmed the direct life cycle of this species which was later worked out in detail by Wehr (1939).

All efforts of various investigators to transmit embryonated eggs of *Capillaria annulata* by direct feeding have been unsuccessful. However, Wehr (1936) showed that earthworms of the species *Helodrilus foetidus* and *Helodrilus caliginosus* serve as intermediate hosts for this parasite.

Due to the evident variation in method of transmission among the capillarids, it would seem unwise to postulate any particular life cycle for a given species. It is apparent that the life cycle must be worked out individually for each species.

EXPERIMENTAL

MATERIAL AND METHODS

The capillaria worm eggs used in these experiments were obtained from infected chickens originating in various parts of the United States. To prepare a culture of eggs, the droppings of one or more infected birds were collected and washed through a fine-mesh milk strainer screen in order to remove the coarse particles. The washings were then allowed to settle and the liquid was decanted, leaving the sediment containing the droppings at the bottom of the beaker. In some cases this process was repeated 2 or 3 times, depending upon the amount of sediment present. A centrifuge equipped with 50 cc. tubes was used to remove excess water from the sediment. The eggs were then suspended in a saturated NaCl solution by centrifugation and the salt water was poured off, diluted to about 5 times its volume with tap water and the eggs were then concentrated in the bottom of a centrifuge tube. By repeating the salt-flotation process a culture of eggs could be obtained which was practically free of fecal material. The methods used for embryonation of capillaria worm eggs have been fully discussed elsewhere in this paper.

For the high temperature experiments an electrically operated, thermostat-controlled incubator was used, and for the low temperature experiments a Crosley household refrigerator was employed.

All chicks used in the transmission experiments were hatched in the laboratory and were kept in wire bottom cages from the time of hatching until the termination of the experiment, the kind used in any given experiment usually being determined by the availability of the birds at that particular time, although a preference for the heavier breeds prevailed since the latter are easier to handle in cages than the Mediterranean breeds.

Unless otherwise noted in the text, the grasshoppers, confused flour beetles, and *Tenebrio molitor* used in the intermediate host tests were laboratory raised. All other arthropods as well as the earthworms were collected from chicken runs and other nearby locations.

The measurements of worms and worm eggs, as recorded in this paper, were made with an ocular micrometer or a camera lucida. All photographs were time exposures taken with the aid of a Bausch and

TABLE 1
GEOGRAPHICAL DISTRIBUTION OF CAPILLARIDS OF THE LOWER DIGESTIVE TRACT

State	County	No. of Birds	<i>C. caudinflata</i>		<i>C. columbae</i>		<i>C. retusa</i>	
			Males	Females	Males	Females	Males	Females
Alabama	Jefferson.....	1					9	20
Georgia	Ware.....	1			11	10		
Illinois	Adams.....	12	121	237	5	11		
	Bond.....	1	11	23				
	Lake.....	1		3	3	15		
	Stark.....	1	7	33				
	Stevenson.....	1	7	3				
Indiana	Jackson.....	1			41	32		
	Sullivan.....	2	1	8				
Iowa	Bremer.....	2	1	4				
	Buena Vista.....	1		1				
	Butler.....	1		3				
	Cerro Gordo.....	1		4				
	Chickasaw.....	2	31	134			2	4
	Floyd.....	6	12	101			1	1
	Hancock.....	2	4	52				
	Howard.....	2	2	1				
	Mitchell.....	2	1	27				
	Muscatine.....	1		4				
Kansas	Palo Alto.....	1		1				
	Brown.....	1	6	11				
Kentucky	Miami.....	1		1				
	Henderson.....	1		1				
Maryland	Wicomico.....	1			16	11		
	Baltimore.....	1			2	45		
Michigan	Bay.....	2	84	158				
	Ottawa.....	1	4	7				
Minnesota	Faribault.....	3		14				
	Freeborn.....	1		8				
	Le Sueur.....	2			38	159		
	McLeod.....	1		2				
	Norman.....	2			116	230		
	Stearns.....	2	2	14				
Missouri	Waseca.....	2			1	10		
	Jefferson.....	1		2				
	Linn.....	1		6				
	Nodaway.....	4	9	12				
	St. Louis.....	?	1	12				
New York	Perry.....	1		2				
	Cortland.....	1		23				
	Otsego.....	1			16	103		
	Wayne.....	1		4				

TABLE 1—Continued

State	County	No. of Birds	<i>C. caudinflata</i>		<i>C. columbae</i>		<i>C. retusa</i>	
			Males	Females	Males	Females	Males	Females
Ohio	Cuyahoga	1	16	85				
	Hardin	1		1				
	Putnam	1		3				
	Richland	1		2		3		
	Shelby	1			1	11		
	Stark	1					1	7
Pennsylvania	Union	1	1			4		
	Somerset	1			95	209		
R. Island	Providence	1				11		
W. Virginia	Marion	1	4	11		2		
Wisconsin	Brown	1				1		
	St. Croix	1			4	13		
	Dunn	1	85	249				
	Trempealeau	1		1	31	49		

Lomb microscope and an Eastman Senior 620 kodak or a view camera using cut film.

The material for the geographical distribution studies was obtained in different ways. The principal source of specimens was through the diagnosis laboratory of Dr. Salsbury's Laboratories which receives chickens from all parts of the United States. A second source of specimens was the purchase of chickens for experimental purposes from various poultry raisers and produce dealers throughout the country. All specimens, whatever the source, were received and identified by the writer.

Microscope slides were made of many specimens using various techniques, but the best results were obtained by placing the worms in glycerine jelly and mounting them between two coverglasses, one of which was about 2 mm. smaller than the other. The coverglasses were then mounted on a microscope slide with the smaller coverglass toward the balsam.

GEOGRAPHICAL DISTRIBUTION STUDY

A preliminary report on the geographical distribution of three species of capillarids, *C. caudinflata*, *C. columbae*, and *C. retusa*, found in the lower digestive tract of chickens was made by the writer (1939). Having continued this study, a complete report is made at this time. One or more capillariid species have been found in chickens received from 56 counties located in 17 different states (Table 1). A total of 1,678 *C. caudinflata*, 1,309 *C. columbae*, and 45 *C. retusa* were collected and identified from approximately 90 naturally infected chickens received at Dr. Salsbury's Laboratories, Charles City, Iowa. Of these, 410

(24.43 per cent) *C. caudinflata*, 380 (29.02 per cent) *C. columbae*, and 13 (28.88 per cent) *C. retusa* were males. The largest number of capillarids found in a single bird was as follows: *C. caudinflata*, 334; *C. columbae*, 346; and *C. retusa*, 29. Six of the birds were host to both *C. caudinflata* and *C. columbae*.

C. caudinflata OVA AND THEIR DEVELOPMENT

The eggs of *Capillaria caudinflata* are spindle-shaped, slightly yellowish in color, and have thick punctate shells provided with a transparent opercular plug at each end. Although there is some variation in the size and shape of the eggs of this species, they are a little narrower in proportion to their length than are the eggs of *Capillaria columbae*. Dujardin (1845) stated that the eggs of *Trichosoma longicolle* (= *C. caudinflata*) measure 61 μ long by 23 μ wide, and Morgan (1932) found the average measurements to be 53 μ long by 23 μ wide. The writer has found that 25 eggs measured by means of an ocular micrometer varied from 50 μ to 59 μ in length and from 21 μ to 24 μ in width. The mean size was 55 μ by 23 μ . All measurements were made without the use of a coverglass in order to avoid distortion of the eggs due to pressure.

Eggs of *Capillaria caudinflata* are passed in an unsegmented condition in the droppings of infested chickens (Plate I, Fig. 1). Early attempts to develop these eggs in 2 per cent formaldehyde solution met with failure. When another bactericide, 2.5 per cent potassium dichromate, was substituted it was found that approximately 100 per cent embryonation could be obtained in the cultures. This appears to be in sharp contrast to the results reported by Levine (1936), who attempted to incubate eggs of the pigeon capillarid in 2 per cent potassium dichromate but found that many of the embryos died before embryonation was completed. Cram (1936) found that weak solutions of formalin and potassium dichromate were unsuitable as embryonating media for capillarid eggs. Although Wehr (1939) reported the use of tap water or distilled water for the embryonation of *Capillaria columbae*, he stated that eggs embryonated in 1 to 2 per cent formalin contained fully formed embryos as early as those cultured in tap or distilled water. The writer has also found this to be true in the case of *C. columbae* whereas eggs of *C. caudinflata* failed to develop properly, thus indicating a greater susceptibility of the latter species to the action of formaldehyde. Cultures of *C. caudinflata* eggs for the early part of this investigation were embryonated in 2.5 per cent potassium dichromate solution, although 1 per cent nitric acid and tap water have been used with good success.

A single comparative test on the value of 2.5 per cent potassium dichromate solution and tap water for use in embryonating *C. caudinflata* eggs was run on a divided culture from one infested bird. Since 94 per cent of the eggs in potassium dichromate solution and 92 per cent of the eggs in tap water became embryonated, there is apparently little difference in the value of these two media.

The contents of freshly passed unsegmented eggs of *C. caudinflata*

have a uniformly granular appearance except for a round or flask-shaped equatorial spot which (Plate I, Fig. 1) is possibly associated with the fertility of the ovum, although it has often been observed in eggs which fail to undergo segmentation.

The incubation period of *C. caudinflata* eggs is somewhat longer than the 6 days required by *C. columbae*. The following observations on the development of *C. caudinflata* eggs were made on a culture collected from droppings passed during a 5-hour period. After these eggs were freed from the droppings by salt flotation and centrifugation, they were placed in petri dishes in a shallow layer of tap water and were held at a room temperature of $78^{\circ} \pm 4^{\circ}\text{F}$. The majority of the eggs had undergone their first cleavage at the end of 24 hours (the incubation time as stated in this report is figured from the beginning of the 5-hour collection period), but none had yet undergone the second cleavage. Not until 5 hours later were any eggs observed in the three-cell stage.

The first and second cleavages which occur very regularly in these eggs are total and unequal. The first cleavage line runs transversely, resulting in a new cell containing a little less than one-third of the contents of the egg (Plate I, Fig. 2). The second cleavage line appears in a similar position toward the opposite end of the egg, resulting in two polar cells of approximately the same size with a somewhat larger cell lying between them (Plate I, Fig. 3). No prediction can be made as to where the third cleavage line will appear. As a rule, the first cell to be formed divides to produce the fourth cell, but in some cases it may be formed by division of the center cell (Plate I, Fig. 4). From this stage of development, cleavage proceeds without any apparent sequence or rhythm.

Examination of eggs 45 hours after the beginning of the collection period showed the majority of them to be in the eight-cell stage although one egg was found to contain 14 cells. At the eight-cell stage the blastomeres are rather large (Plate I, Figs. 5, 6). When the culture was examined after 54 hours of incubation, it was difficult to accurately determine the number of cells. However, the majority of the eggs appeared to contain morulae made up of small blastomeres. A very close estimate of the number in one egg showed that it was in approximately the 20-cell stage.

Up to this time, no characteristic arrangement of the cells within the egg was observed, but as development proceeded beyond the 20-cell stage, a peripheral arrangement of the blastomeres occurred and there was a more or less sharp delimitation of the peripheral cells from a central cluster (Plate I, Fig. 7). This arrangement appeared quite definite in eggs which had been incubated for 70 hours. Thus far, the blastomeres had retained a uniformly granular appearance and had occupied virtually the entire area within the egg shell, but eggs examined after 120 hours of incubation showed a tendency for one end of the embryo to lose its granular appearance, the protoplasm becoming more translucent. At this time, the clear region of the embryo began to shrink

away from the inner surface of the egg shell (Plate I, Fig. 8). The shrinking, which proceeded so slowly that no motion could be observed, produced a space of considerable size between the embryo and the inner surface of the shell. Eggs observed after 173 hours of incubation did not contain actively motile embryos, but the protoplasm at the translucent end had become less granular, and the margin at the clear end had assumed a truncate appearance. The transformation into vermiform embryos then proceeded rapidly. An elongation of the embryo, occurring chiefly at its granular end, again caused the embryo to fill the space within the egg shell. As the embryo continued to elongate, the first indication of active motility was observed. The embryo, somewhat vermiculate in appearance, became successively bean-shaped (Plate I, Fig. 9), S-shaped (Plate I, Fig. 10), and U-shaped. The motility of the embryo for a time was confined largely to the granular end of the larva, but later the entire worm was seen thrashing about within the egg shell. The method whereby the transformation into a vermiform embryo takes place is not quite clear. Elongation of the embryo plays a definite part, but it is also possible that there is a splitting of part of the cells at the granular end, away from the remainder of the embryo. At any rate, a few of the eggs examined after 197 hours of incubation contained U-shaped embryos, while some were still in the bean-shaped stage, others were in the S-shaped stage, and a few had not yet reached any of these developmental stages. Molting of the larvae within the egg shell, used in some species of nematodes to indicate maturity, has not been observed in *C. caudinflata*. However, coiled embryos resembling those which had been kept for several months were observed after 262 hours of incubation (Plate I, Figs. 11, 12).

The above observations show that the eggs of *C. caudinflata*, held in tap water at a room temperature of $78^{\circ} \pm 4^{\circ}\text{F.}$, appear to become fully embryonated in approximately 11 days. The first indication of active motility was observed on the eighth day of incubation. Since it is not known whether the above conditions were optimum for development, it is possible that other conditions might shorten the period of development to some extent. However, experiments which follow show that extremely low temperatures and higher temperatures have an adverse effect upon development.

THE EFFECT OF ENVIRONMENTAL FACTORS ON *C. caudinflata* EGGS

A series of tests was conducted in order to determine the effect of high and low temperatures, and of certain chemicals upon unembryonated and embryonated eggs of *C. caudinflata*.

The eggs used in these experiments were collected in the manner previously described. If tests were to be run on unembryonated eggs, freshly collected cultures were immediately subjected to the environmental condition under consideration. For tests on embryonated eggs, the cultures were held in 2.5 per cent potassium dichromate solution or tap water at room temperature until the embryos became motile.

Data on the effect of high, low, and outdoor winter temperature on unembryonated *C. caudinflata* eggs is recorded in Table 2. The nine cultures used in these experiments were divided into two parts, except as indicated, so that the development of a control culture held at room temperature could be compared to the development of the eggs placed in the incubator or refrigerator.

TABLE 2
EFFECT OF TEMPERATURE ON THE VIABILITY OF UNEMBRYONATED EGGS
OF *Capillaria caudinflata*

Type of Experiment	Exp. No.	Temperature of Exposure	Length of Exposure in Days	Period of Incubation After Exp.	Percentage of Eggs Embryonated	
					Exposed Culture	Control Culture
High Temperature	1.	40° ± 2°C.	107 *	0	0	No control
	2.	40° ± 2°C.	23	0	0	12
	3.	40° ± 2°C.	31	0	0	100
	4.	42° ± 4°C.	42	0	0	100
Low Temperature	1.	-10°C.	14	84	21	96 †
	2.	-10°C.	33	127	58	No control
	3.	6°C.	39	0	8	92
Outdoor Winter Temperature	1.	-21° to 68°F.	44	0	0	94
	2.	-21° to 68°F.	64	0	0	96

* This culture dried up on the 107th day but was moistened and the eggs were examined.

† This record made on the 44th day.

In these experiments, exposure of unembryonated *C. caudinflata* eggs to a temperature of 40° ± 2°C. for periods of 23–107 days proved fatal to all eggs. However, some unembryonated eggs subjected to a temperature of -10°C. for periods of 14–33 days survived the exposure as shown by their development when removed to room temperature. Outdoor temperature varying from -21°F. to 68°F. was fatal to two cultures exposed for 44 and 64 days, respectively.

Unembryonated and embryonated eggs showed a marked difference in their reaction to temperature. The following experiments indicate that low temperature is much more detrimental to embryonated eggs than was shown for unembryonated eggs, but the reverse was true for the high temperature.

Two cultures of embryonated *C. caudinflata* eggs were subjected to a temperature of 40° ± 2°C. for a period of 42 days. No degeneration of the embryos in these cultures could be noted even after a period of 6 weeks following their exposure.

Embryos in a culture of *C. caudinflata* eggs held at -10°C. for 21 days showed no degeneration when first removed to room temperature. When they were examined 150 days later, however, only 21 per cent of them appeared normal. In a control culture which had been held at

room temperature throughout the experiment, 96 per cent of the embryos appeared to be normal.

In another experiment; half of a divided culture was placed on a window ledge outside the laboratory where it remained for 63 days, the other half being maintained at room temperature throughout the experiment. The outside winter temperature variation during this period was -21°F. to 68°F. as recorded by the local federal weather observer. At the end of the exposure period, no degeneration of embryos in either half of the culture could be detected. At the time these observations were made, no method was available for testing the viability or infectivity of the embryos. Therefore, these experiments on the effect of temperature on embryonated eggs may be regarded only as indicative and not as conclusive. Although some damage was undoubtedly caused by the low temperature, the amount of damage could not be estimated with any degree of certainty.

Later in the investigation the writer discovered that earthworm digestive juice would produce motility in larvae of embryonated *C. caudinflata* eggs and would cause many of them to hatch. This phenomenon was utilized in the following experiment for testing the viability of embryonated eggs subjected to low temperature.

Eggs from 17-day-old *C. caudinflata* culture were treated with freshly collected earthworm digestive juice. Hatching of many larvae followed after several minutes. On the following day the culture was divided into three parts. Part 136A was placed in the ice tray of an electric refrigerator where the temperature was -10°C. ; part 136B was placed in the bottom of the refrigerator at a temperature of 6°C. ; and part 136C was held at room temperature. Six days later each culture was tested for viability by treatment with earthworm digestive juice. Hatching occurred in all three samples. On the 14th day of refrigeration, eggs held in the freezing tray failed to become motile or to hatch although many larvae hatched from parts 136B and 136C when treated with the same sample of earthworm digestive juice. The same results were obtained when the three cultures were treated with fresh samples of earthworm digestive juice on the 2 succeeding days. Thus the larvae were able to survive periods of 6 days at -10°C. and 14 days at 6°C. but were killed by 14 days exposure at -10°C.

Two per cent formalin has proven harmful to *C. caudinflata* eggs. In one experiment, only 18 per cent of the eggs in 2 per cent formalin developed to the larval stage when held at room temperature for 5 months, whereas the control culture in 2.5 per cent potassium dichromate solution showed 100 per cent embryonation. In another experiment a culture was divided into three parts, one part being placed in tap water, a second part in 1 per cent formalin solution, and the third part in 2 per cent formalin solution. After 42 days, 92 per cent of the eggs in tap water contained coiled embryos, whereas none of the eggs in 1 per cent formalin had developed further than the five-cell stage and none in 2 per cent formalin solution had passed the two-cell stage, 96 per cent of the eggs in the latter culture showing no cleavage lines at all.

THE LIFE CYCLE OF *C. caudinflata**Experiments on Direct Transmission of C. caudinflata*

Numerous experiments have been conducted in order to test the possibility of transmitting *C. caudinflata* by feeding embryonated eggs directly to chickens. Twenty-one chicks used in various experiments failed to become infected by feeding large single doses of *C. caudinflata* eggs which had been embryonated in 2.5 per cent potassium dichromate solution, 1 per cent nitric acid, or tap water. Since repeated doses of embryonated eggs administered to individual chicks produced no better results, a culture was held at 6°C. for 75 days in the hope that low temperature might in some way render the eggs infective. Repeated doses of these refrigerated eggs, however, were non-infective to two chicks. Other tests which gave negative results were (1) repeated passage of a single culture through a chick and (2) the administration of eggs which had been exposed for 10 days at 35°C. to the filtered digestive juices obtained from chicken gizzard and duodenum.

Since it appeared that chickens could not be infected with *C. caudinflata* by direct administration of embryonated eggs, an attempt was made to hatch the larvae and produce infection with them. It was found that many of the larvae in partially desiccated cultures of *C. caudinflata* eggs would hatch when the eggs were returned to water. Larvae hatched in this manner were given to six chicks but failed to produce infection in any of them. A further unsuccessful attempt at transmission to a chick was made by feeding samples of soil to which many embryonated eggs had been added 32 days previously. A repetition of this experiment likewise failed to produce infection.

In order to provide continuous exposure of chicks to *C. caudinflata* eggs, where little or no chance for infection through some unknown intermediate host would be available, a pen 3 feet \times 4 feet was constructed in a brooder house where no chickens other than those used in the experiment were kept. A 3-inch layer of soil which had been sterilized in an autoclave was placed in the bottom of the small pen. This soil was kept moist throughout the test. Four chicks confined for periods of 2.5 to 6 months in this pen with an adult bird passing eggs of *C. caudinflata*, did not become infected.

A further attempt was made to establish a *C. caudinflata* infection in a chick by introducing approximately 400 freshly collected living adult capillarids directly into the crop of a chick. Post-mortem examination 45 days later showed that none of the worms had become established in the intestine.

Experiments on Indirect Transmission of C. caudinflata

Since all experiments on direct transmission of *C. caudinflata* gave negative results, the writer conducted experiments attempting to discover an intermediate host for this nematode. Arthropods used in these experiments without success included the grasshoppers *Melanoplus differentialis*, *Melanoplus femur-rubrum*, and an undetermined species

of green meadow grasshopper belonging to the family Tettigoniidae; the beetles *Tenebrio molitor*, *Tenebroides mauritanicus*, *Tribolium confusum*, and *Aphodius* sp.; housefly larvae and adults; undetermined species of reddish-brown ants; and an unknown species of sow-bugs.

Since earthworms are known to serve as transmitting agents for the gapeworm *Syngamus trachea* and the crop-worm *Capillaria annulata*, it seemed quite possible that they might also serve for the transmission of *C. caudinflata*. During the summer of 1939 and the spring of 1940, earthworms of the species *Lumbricus terrestris* and another smaller species, *Helodrilus* sp., were used in several experiments on the transmission of *C. caudinflata*, but none of the chicks became infected. No further attempts were made to use earthworms until late in 1941.

In order to build up a heavy population of capillaria worm eggs in a small area and thus increase the chances of transmission to chicks, two outdoor pens approximately 10 feet square were constructed. On April 30, 1941, twelve 54-day-old battery-raised chicks were placed in each of these pens together with two or three adult infected hens. Each chick was checked by salt-flotation in order to show that its droppings were free of capillaria eggs. Salt-flotation examinations of the droppings from each chick were made weekly for the following 25 weeks. On three occasions, capillaria eggs were observed, but subsequent flotations failed to show more eggs, indicating contamination by droppings from the adult hens. One chick was killed two days after a capillaria egg was observed but no capillarids could be found on post-mortem. Transmission was not accomplished in five other chicks which died during the 25-week period. When the surviving test chicks and the adult hens were sacrificed, within 30 days following the termination of the fecal examinations, one of the test chicks was host to one male and one female *Capillaria retusa*, and another had one female *C. caudinflata*. None of the other test chicks was infected although both species of capillarids were found in the adult hens.

Since one chicken of the preceding experiment became infected with *C. caudinflata* while in the experimental pen, earthworms, dung beetles (*Aphodius* sp.), and house-fly larvae were collected from the two pens and fed to chickens. Approximately 100 earthworms were given to each of four chicks during the period Oct. 23, 1941, to Nov. 3, 1941. Examination of droppings from these chicks on Nov. 31, 1941, showed that all four of them had become infected with capillarids. Worms identified as *Capillaria caudinflata* were obtained by post-mortem from three of the four chicks. One male capillarid identified as *C. retusa* was recovered from the cecum of one of the chicks. Forty-four dung beetles and seven house-fly larvae failed to produce *C. caudinflata* infection when fed to chicks.

In order to determine whether earthworms serve as true intermediate hosts or as incidental hosts for *C. caudinflata*, the following experiment was carried out. On December 4, 1941, about 250 earthworms identified as *Helodrilus* (*Allolobophora*) *caliginosus*¹ were collected from

¹ Identification confirmed through the courtesy of Dr. Benjamin Schwartz, Chief, Zoological Division, Bureau of Animal Industry, U.S.D.A., Washington, D. C.

a city lot where no chickens had run for many years. These worms were placed in a wooden box about $8 \times 8 \times 4$ inches, and fresh cultures of *C. caudinflata* eggs were poured on the surface of the soil in this box from time to time.

Thirty-seven days after the earthworms were first exposed to the capillaria eggs, a 7-weeks-old New Hampshire chick, No. 763, received five earthworms from this box. Fifteen days later a second chick of the same hatch, No. 940, received 12 earthworms from the box, and 12 days later a third chick, No. 969, received 20 earthworms. One New Hampshire chick, No. 928, was given 10 grams of soil from the box in which the above earthworms were kept. This bird served as a control against the possibility of the earthworms acting merely as mechanical carriers for capillaria larvae. Chicks Nos. 942 and 947 received neither earthworms nor soil but were used as "cage controls" against the possibility of the chicks acquiring an infection in some unknown manner.

Chick No. 763, which died on February 2, 1942, 23 days after receiving the earthworms, was host to two male and five female capillarids identified as *C. caudinflata*. Capillaria eggs were found in the droppings of chicks Nos. 940 and 969 when they were examined by the centrifuge salt flotation method on the twenty-fourth day after receiving earthworms. Chick No. 940 had apparently lost its infection by May 11, since no capillaria worms could be found at post-mortem. Chick No. 969, however, was host to 5 male and 12 female worms identified as *C. caudinflata*. Post-mortem examination of control chicks Nos. 928, 942, and 947 failed to reveal the presence of any capillaria worms.

The results of this test further indicated that *Capillaria caudinflata* was transmitted by earthworms belonging to the species *H. (A.) caliginosus*.

Ten earthworms of the species *H. (A.) caliginosus* collected in the fall and held in a box of soil for about 4 months were given $\frac{1}{4}$ to $\frac{1}{2}$ cc. of a 66-day-old culture of *Capillaria caudinflata* eggs. The eggs were forced into the digestive tract of the earthworms by means of a capillary pipette inserted into the mouth. Each drop of the culture contained approximately 15 embryonated eggs. These earthworms were kept in a small jar of soil until March 4, 1941, when four surviving worms were given to an 8-weeks-old New Hampshire chick, No. 11.

Two days after the earthworms were given to this chick, three chicks of the same breed and age were treated as follows: chick No. 12 was given approximately 750 embryonated eggs from a 33-35-day-old culture; chick No. 13 was given 10 earthworms which had never received any capillaria eggs; and chick No. 14 received neither capillaria eggs nor earthworms thus serving as a cage control. All the chicks were placed in one cage.

When droppings from each of the chicks were examined by the centrifuge salt-flotation method on the twenty-third and twenty-fourth days (twenty-fifth and twenty-sixth for No. 11) no capillaria eggs were found. These chicks were killed during the next two days and the digestive tracts thoroughly examined for capillaria worms. One male

C. caudinflata was recovered from chick No. 11 on April 9, 1942. All other chicks failed to become infected.

There are three possible ways in which chickens receiving earthworms might acquire a *C. caudinflata* infection. First, the earthworms might serve as true intermediate hosts; second, the earthworms might serve merely as mechanical carriers of infective larvae developing in the soil where the earthworms were raised; and third, the chicks might acquire the infection independently of the earthworms while confined in the test cages. Therefore, the following experiment was carried out in order to determine which of these methods was correct.

Several hundred earthworms identified as *H. (A.) caliginosus* were collected in a field more than $\frac{1}{4}$ mile from any poultry yard. Hence, it is believed that the soil had no chance for contamination by poultry droppings containing capillaria worm eggs. These earthworms were placed in a clean box of uncontaminated soil and on the following day, freshly collected eggs of *C. caudinflata* were placed on the surface of the soil. Cultures were added every second day until three cultures containing approximately 12,000 eggs each had been applied.

On April 30, 1942, the thirty-eighth day after the first culture of capillaria eggs was given, 10 Barred Rock chicks 44 days old were each fed 12 of the above earthworms. Each of 10 chicks from the same hatch received 10 grams of soil from the box of infected earthworms, the soil being shaped into pellets and forced into the crop; a third group of 10 chicks received no treatment other than regular feed and water and thus served as cage controls against any chance infection from unknown sources; and each of the 10 chicks in the fourth group was given approximately 600 embryonated eggs from a 52-day-old culture. When one battery-raised chick received earthworms to which eggs from this 52-day-old culture had been administered by pipette, it became infected with *C. caudinflata*, proving the infectivity of this culture.

Droppings of the 10 chicks receiving earthworms were positive for capillaria eggs by May 24, 1942, as shown by the centrifuge salt flotation method. These chicks were killed, and post-mortem examination was made. After the intestine of each chick was removed and the ceca severed for separate examination, the intestine was slit open, and the food material was removed and washed through a 100-mesh screen in order to eliminate the fine food particles. The remaining substance was then examined with the aid of a 2.25 power Zeis binocular head-band magnifier. Small portions of the food substance were suspended in water using a large pyrex bake dish resting on a black background.

The entire lining of the intestine from the gizzard to the cloacal orifice was then scraped from the intestine, washed through the same screen and examined in the manner just described. From these chicks, 275 *C. caudinflata* worms were recovered, 83 of which were males. The least number of capillarids found in any of the 10 chicks was 7 while the maximum number was 58. Each chick in the three control groups was killed and carefully examined in the same manner, but no capillaria worms were found in any of the 30 control chicks.

From this and previous experiments it is concluded that earthworms of the species *H. (A.) caliginosus* serve as true intermediate hosts for the intestinal capillarid, *C. caudinflata*.

In order to prove that earthworms transmitting *C. caudinflata* to chickens actually acquire their infection after being brought into the laboratory, another experiment was carried out.

About 350 earthworms identified as *H. (A.) caliginosus* were collected from a heavily wooded lot approximately $\frac{1}{4}$ mile from the nearest poultry yard. These worms were divided into 2 equal groups on June 20, 1942, and were placed in clean wooden boxes. One group was held as controls while embryonated *C. caudinflata* eggs were scattered on the surface of the soil in the other box. On July 19, 1942, 12 earthworms from the control box were fed to each of 12, 37-day-old battery-raised New Hampshire chicks, Nos. 3802-3813 inclusive; 12 earthworms from the infected group were given to each of 12 chicks, Nos. 3814-3825. These chicks were of the same breed and same age as the controls. All 24 chicks were kept in wire-floored cages. On August 12, 1942, it was found by salt-flotation examination that all 12 of the chicks receiving infected earthworms were passing capillaria eggs. The droppings of the 12 chicks receiving uninfected earthworms were negative. On August 12 and 13, all the control chicks were killed and careful examination of the intestine was made by the method described in the preceding experiment. No capillaria worms could be found. On August 13 when one of the infected chicks, No. 3814, was killed, 7 male and 10 female *C. caudinflata* were recovered. When the other infected chicks were killed about 3 weeks later, the following numbers of *C. caudinflata* were obtained: No. 3815, 3 females; No. 3816, 4 females; No. 3817, 3 males and 9 females; No. 3818, 5 males and 12 females; No. 3819, 5 males and 9 females; No. 3820, 7 males and 20 females; No. 3821, 16 males and 19 females; No. 3822, 2 males and 11 females; No. 3823, 10 males and 13 females; No. 3824, 2 males and 4 females; and No. 3825, 6 males and 6 females.

Since all of the chickens receiving infected earthworms became parasitized by capillaria worms whereas no capillarids were found in the chicks receiving uninfected earthworms, it may be safely assumed that the earthworms used had no previous infection but were experimentally infected in the laboratory. Since the control chicks did not become infected it should be noted that they also served the purpose of cage controls against infection from unknown sources while the chicks were in the cages.

Developmental Periods of C. caudinflata

PERIOD OF EMBRYONATION

It was discovered that *C. caudinflata* eggs would hatch *in vitro* when exposed to earthworm digestive juice. Tap water forced into the oral cavity of earthworms by means of a capillary pipette was used to flush the entire digestive tract. The contents emerging from the anus were

collected and filtered to remove debris. When a few drops of this filtrate were placed on a microscope slide with a drop of culture containing numerous embryonated eggs, the larvae became very active, and in 2 to 5 minutes most of them hatched. This method of hatching provided a valuable tool for the determination of maturity in *C. caudinflata* eggs, and was used in the experiment which follows.

A *C. caudinflata* egg culture was collected from droppings passed by several infected chicks during a 5-hour period. Since most of the eggs contained motile embryos by the tenth day, a few drops of the culture were treated with freshly collected earthworm digestive juice. None of the larvae hatched. However, the same sample of earthworm digestive juice produced hatching in a 35-day-old culture, thereby proving the activity of the digestive juice sample. On the eleventh day, the eggs failed to hatch even after 22 hours exposure, whereas eggs from the 36-day-old control culture hatched within a few minutes. Although the digestive juice appeared to stimulate the worms to greater motility, the larvae were unable to rupture the egg. Similar treatment on the twelfth day caused three larvae to hatch. On the thirteenth day about 25 larvae had hatched within 1 hour after treatment, thus indicating a much higher percentage of maturity than was present on the preceding day.

Since the *C. caudinflata* eggs in this *in vitro* experiment were capable of hatching after a 12-day embryonation period, it is probable that the embryonation was complete and that the larvae would be infective to earthworms.

DEVELOPMENTAL PERIOD IN THE EARTHWORM

A series of experiments was carried out in order to determine the shortest developmental period required in the earthworm before *C. caudinflata* becomes infective to chickens. Earthworms for these experiments were collected from localities where it was unlikely that they would be naturally infected with capillarids. The earthworms for each experiment were divided into two lots, one lot receiving embryonated capillaria eggs, the other being held for feeding to control chicks. Earthworms from the infected lot were given to chicks at varying intervals after receiving the capillaria eggs, while a similar number of earthworms from the uninfected lot was given to a control chick.

In one experiment, three chicks received earthworms which had been exposed to embryonated eggs for 11, 15, and 21 days, respectively. All three chicks became infected with *C. caudinflata*, whereas the chick receiving earthworms from the control lot remained uninfected.

In a second experiment, earthworms which had been infected, by means of a capillary pipette, with embryonated eggs 9 days previously were able to transmit the infection to a chick. A chick receiving uninfected earthworms from the control lot did not become infected with capillarids.

In a third experiment, five chicks each received 40 to 50 earthworms

which had been infected by means of a pipette, with embryonated eggs. The respective lots of earthworms given to these five chicks had received embryonated eggs 1, 3, 5, 7, and 9 days before they were given to the chicks. *C. caudinflata* worms were found in the chick receiving earthworms infected for 9 days, but no capillarids were obtained from the other chicks. A control chick which had received 50 uninfected earthworms did not become infected with capillarids. In all the experiments conducted, 9 days in the earthworm was the shortest developmental period which has produced *C. caudinflata* larvae infective to chickens.

DEVELOPMENTAL PERIOD IN THE CHICKEN

Observations were made on 14 experimentally infected chicks in order to determine the length of time required before the female *C. caudinflata* worms become mature. Maturity was indicated by the appearance of eggs in the droppings.

Centrifuge salt flotations were run on the droppings of each of the 14 chicks on 1 to 5 consecutive days immediately preceding the appearance of eggs in the droppings. Eggs were found on the twenty-second day in four of the chicks, on the twenty-third day in eight of them, and on the twenty-fourth day in two of them. In each case, the identity of the capillarids was proven by post-mortem examination of the chicks.

The writer has examined many other chicks following experimental infection with *C. caudinflata*. In no case where female worms were present have the eggs failed to appear by the twenty-fourth day.

Description of C. caudinflata Developmental Forms

THE EMBRYOS

Hatching, initiated by the application of earthworm digestive juices to embryonated eggs, produced abundant material for morphological study of the embryos. After hatching, the larvae were usually allowed to remain on the slide until they became quiescent, or they were heated gently over an open flame. It was found that a little acetic acid added to a weak alcoholic solution of iodine was very satisfactory for killing capillariid embryos and often helped to differentiate some structures.

The newly hatched first-stage larvae (Plate I, Fig. 13, and Plate II, Fig. 1) were approximately .175 mm. long and .0086 mm. wide (Table 3). These larvae, after freeing themselves from the egg shell, moved slowly back and forth. The anterior end was most active, having a halting exploratory motion. This characteristic, carried over to the larvae occurring in the earthworm, was very helpful in their identification since the action was quite unlike that of rhabditids or other nematode parasites observed in earthworms. In some embryos, a stylet was seen protruding from the anterior end. The posterior end was bilobed. In these larvae, the oesophagus was quite well differentiated, but the intestine was not well defined. The cell body, which appears as a column of irregularly shaped cells, measured about .055 mm. in length.

LARVAE FOUND IN THE EARTHWORM

No moulting forms have been recovered from earthworms, although the considerable growth and development which occurs in the earthworm and the fact that repeated attempts to infect chickens directly with embryonated eggs have proven unsuccessful, suggest that the embryos must have moulted at least once.

Since developmental periods of 15 days in the earthworm have consistently produced infective forms, the larvae used for morphological studies were obtained from earthworms which had been infected for 15 days or more. The second-stage larvae were obtained for observation in the following manner. Infected earthworms were placed in a petri dish, submerged in a little water and slit open with a pair of scissors. The internal structures were then scraped from the body wall and the macerated tissues examined under a wide-field binocular microscope, using a magnification of about $100\times$. The larvae were usually found free of debris, resting on the bottom of the dish.

TABLE 3
MEAN MEASUREMENTS OF *Capillaria caudinflata* LARVAE AND ADULTS*
(All Measurements in Millimeters)

Stage of Development	Number of Worms	Mean Length	Mean Width	Mean Length of Oesophagus	Mean Length of Intestine	Distance Ant. End to Vulva	Length of Spicule
Embryos.....	10	.175	.0086	.063	.112
Larvae from earthworms.....	10	.183	.0119	.070	.113
Larvae in chick (5 day).....	5	.552	.0228
Larvae in chick (9 day).....	1	1.160	.0280	.900	.260
Larvae in chick (11 day).....	2	1.560	.0270	1.180	.380
Larvae in chick (12 day).....	2	4.320	.0339	2.365	1.955
Immature females (12 day).....	2	5.295	.0384	2.950	2.345
Immature female (13 day).....	1	8.300	.0400	3.640	4.660
Immature females (17 day).....	2	15.835	.0539	6.155	9.680
Immature females (19 day).....	2	19.650	.0679	6.225	13.425
Immature male (12 day).....	1	3.430	.0310
Immature males (15 day).....	2	7.520	.0392	3.785	3.735
Immature males (17 day).....	2	9.425	.0399	4.305	5.120
Immature males (19 day).....	2	12.685	.0483	5.780	6.905
Adult females.....	10	18.960	.0693	6.470	12.490	6.580
Adult males.....	10	13.970	.0587	6.210	7.760	1.140

* The individual measurements from which these figures were obtained are recorded in the original thesis.

These capillaria larvae were approximately .183 mm. long and .0119 mm. wide (Table 3). The posterior end, which bears a membranous bilobed bursa-like structure, was narrowly rounded (Plate I, Fig. 14, and Plate II, Fig. 2). No stylet could be observed. The oesophagus was more clearly defined than in the first-stage larva, and a definite intestine could be clearly seen posterior to the cell body. The cell body measured about .061 mm. At either end of the latter structure irregular-shaped cells could be seen in the coelomic cavity.

LARVAE FOUND IN THE CHICKEN

When infected earthworms are eaten by a chicken and the *C. caudinflata* larvae start to grow, development is confined chiefly to the anterior region for the first few days.

Five *C. caudinflata* larvae recovered on the fifth day of development in the chicken (Plate I, Fig. 15, and Plate II, Fig. 3) averaged .552 mm. in length and .0228 mm. in width (Table 3). The ratio of the length of the oesophagus to that of the intestinal length was about 3:1. Although no moulting larvae were found at this stage of development, it is probable that part of them had moulted, since two of the five larvae examined retained the membranous bilobed bursa-like posterior appendage characteristic of all larvae examined from the earthworm, while this structure was lacking in three of the worms. The oesophageal-intestinal junction was clearly visible in four of these larvae. The intestine is better differentiated in the 5-day larvae than in those obtained from the earthworm, but there has been only a slight increase in its length. The oesophagus, however, is approximately eight times as long as it was in the larvae from the earthworm. No bacillary bands were observed.

Measurements of one 7-day larva gave a length of .8 mm. and a width of .0281 mm. The oesophageal-intestinal ratio was not obtained, since the junction could not be detected with certainty in this particular specimen. The bilobed posterior membrane observed in some of the 5-day larvae was absent. Thus, the larva resembled the 9-day larva except for its smaller size.

A larva collected on the ninth day, i.e., 9 days after it was eaten by a chicken, had a length of 1.16 mm., the ratio of the length of the oesophagus to that of the intestine being approximately 9:2. The maximum width was .028 mm. (Table 3). At this stage of development, the oesophagus appeared as a small tube running through the cell body (Plate II, Fig. 5). The latter structure, however, was not yet completely organized. The region posterior to the oesophageal-intestinal junction showed only slight development beyond that of the form found in the earthworm. Small refractile bodies present in this area were interpreted as early stages in the development of bacillary bands.

The mean length of two 11-day larvae was 1.56 mm. and the mean width .027 mm. (Table 3). The ratio of the length of the oesophagus to that of the intestine was about 11:4. The principal development over the 9-day larva was an elongation of both the oesophageal and intestinal region, the latter region showing a large number of vacuoles.

Five larvae were obtained from a chicken 12 days after it had received infected earthworms. In three of the five larvae the sex could be determined, although sexual differentiation was by no means complete.

The mean length and width of two female larvae was 5.295 mm. and .0384 mm., respectively (Table 3). The ratio of the length of the oesophagus to that of the intestine was about 1:1. No vulva or ovijector was present in these worms. However, a thick-walled vagina, slightly posterior to the oesophageal-intestinal junction could be clearly seen. In the anterior region, the oesophagus and the cell body were well developed, as were two gland cells at the junction of the oesophagus and the intestine (Plate II, Fig. 4). The bacillary bands were well formed in these larvae.

One of the larvae, believed to be an immature male, had a length of 3.43 mm. and a width of .031 mm. (Table 3). This worm was considered to be a male because of the shape of the caudal end (Plate I, Fig. 17) and the presence of a structure resembling a spicule. The junction of the oesophagus with the intestine could not be observed.

Two sexually undifferentiated 12-day larvae averaged 4.32 mm. in length and .0339 mm. in width, (Table 3). The oesophageal-intestinal ratio was about 2.3:1.9. These worms resembled the 11-day larvae in appearance except that the intestine was proportionally longer. One of these larvae was in the process of moulting (Plate I, Fig. 16), but no visible sexual differentiation was apparent in this larva.

Growth in the posterior region of the larvae proceeded rapidly after the twelfth day. A young 13-day female (Plate I, Fig. 23) measured 8.30 mm. in length and .04 mm. in width (Table 3). The ratio of the length of the oesophagus to that of the intestine was about 1:1.3. Little difference in appearance between this larva and the 12-day female larvae was noted. The reproductive organs were perhaps more plainly visible, but like the 12-day female, no genital opening or ovijector was present.

Two immature male worms collected on the fifteenth day of development in the chicken averaged 7.52 mm. in length and .0392 mm. in width (Table 3). Thus these worms were more than twice as long as the young male recovered on the twelfth day of development. The ratio of the oesophageal length to that of the intestine was about 1:1. In these worms, the oesophageal-intestinal junction was clearly differentiated. No definite bursa-like membrane or alae were present, but the caudal end was somewhat inflated, and it appeared that the two processes which later support the bursal membrane of the adult were beginning to make their appearance.

None of the four male *C. caudinflata* worms observed on the seventeenth day of development in the chicken possessed the bursa-like membrane or the caudal alae characteristic of adult males of this species. The caudal extremity in these worms, as seen in lateral view, showed an enlarged asymmetrical bulblike structure approximately 1.25 to 1.5 times as wide as the body diameter. Two males averaged 9.425 mm. in length and .0399 mm. in width. The oesophageal-intestinal ratio was about 1:1.2.

Although ovijectors were present on only four of the eight females observed on the seventeenth day, definite genital openings could be seen on the worms without ovijectors. It would be very difficult to differentiate these immature females from capillarids of the species *C. columbae*. Therefore, the writer wishes to emphasize the necessity for working with adult worms when attempting specific identification. Maturity can be determined by the presence of eggs in the uterus since it has been determined that the ovijectors are formed before eggs develop. Two females averaged 15.835 mm. in length and .0539 mm. in width. The oesophageal-intestinal ratio was about 1:1.6.

Two 19-day-old male *C. caudinflata* worms were observed and measured. The characteristic bursa-like membrane supported by two T-shaped processes was present in both males, but caudal alae could be seen in only one of them. Even in this specimen the alae had not yet reached adult proportions. These males averaged 12.685 mm. in length and .0483 mm. in width. The oesophageal-intestinal ratio was about 1:1.2.

The development was practically completed in seven 19-day-old female specimens observed. Partially formed eggs could be seen in the oviducts, but they were not yet enclosed in shells. The average length of the two females measured was 19.65 mm. and the average width was .0679 mm. The length of these worms exceeded the average length of 10 adult female specimens measured (Table 3). The oesophageal-intestinal ratio was about 1:2.1.

THE ADULTS

Little need be added to the description and figures of adult *C. caudinflata* worms given by Shipley (1909) and Morgan (1932). The adults are small slender threadlike worms, very difficult to observe until they are removed from the intestine. The anterior portion consists of an oesophagus and cell body while the posterior portion contains the intestine and the genital organs. At the oesophageal-intestinal junction two oval or triangular glandular cells may be seen. The cuticula is faintly striated. A pair of lateral bacillary bands lie just beneath the surface and are very difficult to observe in many specimens (Plate I, Fig. 27).

The females varied from 15.8 to 21.8 mm. long and averaged about .0693 mm. wide (Table 3). The oesophageal-intestinal ratio was about 1:1.9. The females of this species are most easily recognized by the presence of a funnel-like appendage of the vulva which is located just posterior to the cell body (Plate I, Figs. 22, 24, 25, 26). This structure, which was found in all adult females, is a definite structure and not an inflation of the outer wall caused by endosmosis or a prolapsed sheath as suggested by Eberth (1863). Leading to the vulva is a thick-walled vagina, a uterus, and simple ovary consisting of a single tube. The internal structure is difficult to observe because it is usually masked by the presence of numerous brownish yellow eggs. The posterior end of the female is rounded, with the anus subterminal.

The males of *C. caudinflata* are generally much smaller than the females. Ten males varied from 10.3 to 20.3 mm. in length and averaged .0587 mm. wide. The oesophageal-intestinal ratio is about 1:1.2. These males may be distinguished from males of other species found in chickens by the presence of lateral caudal alae and a heart-shaped bursa-like membrane which terminates the posterior end (Plate I, Figs. 18, 19, 20, 21). Two T-shaped processes support the latter structure. The spicule is slender, varying from 0.85 to 1.7 mm. in length. The spicular sheath bears no spines but is marked by transverse striations.

THE LOCATION OF *C. caudinflata* IN THE DIGESTIVE TRACT OF CHICKENS

Records were obtained on the location of *C. caudinflata* in the digestive tract of 25 chickens and 1 turkey. When each chick was killed, the entire digestive tract was removed for examination. In no case were worms found anterior to the small intestine. The lower digestive tract was divided into five portions. The first portion, the duodenal loop, constituted approximately one-fifth of the lower digestive tract, the remainder of the lower digestive tract being divided into four parts. The ceca, the duodenum, and each of these four parts were examined separately for capillaria worms, according to the manner described elsewhere in this paper.

Of 551 worms recovered from the chicks, 314 (56.99 per cent) were in the upper one-fifth (duodenal loop); 182 (33.03 per cent) were in the second fifth; 51 (9.25 per cent) in the third fifth; and 4 (0.73 per cent) in the fourth fifth. No capillarids were found in the last fifth or in the ceca. Of 238 capillarids from the turkey, 126 (52.95 per cent) were in the duodenal loop; 109 (45.79 per cent) in the second fifth, and 3 (1.26 per cent) in the third fifth. No capillarids were found in the fourth, or fifth portions in the ceca. The proportion of male worms found in the turkey was 34.45 per cent, whereas the proportion of males in the 25 chicks was 31.95 per cent.

From these data it may be concluded that *Capillaria caudinflata* is essentially a parasite of the upper half of the small intestine of chickens and turkeys and is particularly abundant in the duodenal loop.

OBSERVATIONS CONCERNING HOST-SPECIFICITY IN *C. caudinflata*

The literature regarding *Capillaria caudinflata* would seem to indicate great freedom so far as host-specificity in this species is concerned. Due to the inadequate descriptions by earlier workers, it is impossible to draw up an accurate host list, although it appears that this capillarid has been found in the following gallinaceous birds: the European quail, *Perdix coturnix*; the common fowl, *Gallus domesticus*; the red grouse, *Lagopus scoticus*; the pheasant, *Phasianus colchicus*; the golden pheasant, *Chrysolophus pictus*; the black grouse or grey-hen, *Lyrurus tetrix*; the capercaillie, *Tetrao urogallus*; the partridges, *Perdix perdix* and *Perdix cinerea*; and the ptarmigan, *Lagopus lagopus*.

In order to help clarify our knowledge of host-specificity, the writer

attempted to determine the susceptibility of turkeys, ducks, and pigeons to infection with *C. caudinflata*.

On April 21, 1942, 11 earthworms of the species *H. (A.) caliginosus*, having been exposed to a 21-day-old culture of embryonated *C. caudinflata* eggs 25 days previously, were fed to a 15-day-old battery-raised, broad-breasted, bronze poult, No. 4950. Another poult, No. 4967, received 11 earthworms of the same origin, but these earthworms had never been exposed to *C. caudinflata* worm eggs. A third poult, No. 4955, received no earthworms and was held as a cage control.

Capillaria worm eggs were present when the droppings of poult No. 4950 were examined by centrifuge salt flotation 23 days after the earthworms were given, but no eggs could be found in the droppings of the controls. The three poults were killed on May 16, 1942. Poult No. 4950 was host to 82 male and 156 female *C. caudinflata* worms, but no capillarids were found in the control poults Nos. 4955 and 4967. This is the first time that *C. caudinflata* has been transmitted to turkeys under controlled conditions, using experimentally infected earthworms as transmitting agents.

An English sparrow, *Passer domesticus*, received 10 earthworms of the species *H. (A.) caliginosus* during a 3-day period. These earthworms had previously received embryonated eggs of *C. caudinflata*. Chicks which received earthworms from this same lot became infected. Since no capillaria worm eggs appeared in the droppings by the thirty-fourth day, the sparrow was killed, and post-mortem examination was made. One mature male and two mature female capillarids recovered from this bird on October 8, 1942, were identified as *C. caudinflata*. Several immature specimens were also found. The mature females had an ovijector typical of *C. caudinflata* although no eggs were present in the reproductive organs, thus accounting for the absence of eggs in the droppings. The mature male had the typical heart-shaped, bursa-like membrane and caudal alae characteristic of *C. caudinflata* (Plate I, Fig. 19).

Five adult English sparrows obtained from a nearby poultry house were used in a second transmission experiment. On Oct. 9, 1942, two of these sparrows received earthworms, *H. (A.) caliginosus*, which had been infected with *C. caudinflata*. The other three sparrows were held as cage controls. When one sparrow was killed on Nov. 13, 1942, 12 male and 20 female *C. caudinflata* worms were recovered. Four of the males and five of the females were immature since caudal alae and the bursal membrane of the males were lacking and no ovijector was present in the females. The other worms appeared to be mature except that no fully formed eggs were present in the uteri of the females.

Salt flotation or centrifuge salt flotation examinations were made daily on the droppings of the other infected sparrow from the thirty-third day after earthworms were given until the bird was killed. No capillaria worm eggs appeared in the droppings until Nov. 20. The sparrow was killed immediately and the capillaria worms recovered. Of the 14 male and 20 female *C. caudinflata* worms present in this sparrow, one male and

five females were immature. Only two of the females contained fully formed eggs in their uteri. No capillarids were obtained from the three controls at post-mortem.

It is probable that the English sparrow is not a normal host for *C. caudinflata* because (1) capillarid specimens recovered from sparrows were somewhat smaller than those commonly found in chickens, and (2) egg production did not begin until the capillarids had developed 41 days in the sparrow whereas the corresponding period in chickens has never been found to exceed 24 days.

The results of these experiments lead the writer to believe that he has successfully transmitted *C. caudinflata* to sparrows, using earthworms of the species *H. (A.) caliginosus* as intermediate hosts. There is a remote possibility that the birds may have been naturally infected prior to the experiment, but this seems unlikely since repeated examination of the droppings revealed no capillaria eggs which could not be attributed to the experimental infection. Furthermore, the fact that both sparrows receiving earthworms became infected whereas none of the three controls was a host to capillarids also indicates experimental infection.

These experiments constitute the first record of *C. caudinflata* infection in a passerine bird. Having thus shown that this species may develop in birds belonging to two different orders, the Galliformes and Passeriformes, it seemed quite possible that birds of other orders might also serve as hosts. Therefore, the writer attempted to infect a White Pekin duck and a White King pigeon with *C. caudinflata* by feeding them infected earthworms of the species *H. (A.) caliginosus*. Although control chicks receiving earthworms at the same time became infected with *C. caudinflata*, neither the duck nor the pigeon became infected. It is possible that repetition of these experiments might meet with greater success.

OBSERVATIONS ON THE LONGEVITY OF *C. caudinflata*

The maximum length of time which unhatched embryonated eggs of *C. caudinflata* may survive, the length of time this species may live in the earthworm, or the maximum age of adults in the chicken has not been determined. However, certain observations have been made which indicate that this species is long-lived in the egg stage and possibly in the earthworm, whereas the length of life in the chicken, at least in some cases, is comparatively short.

Several cultures of *C. caudinflata* eggs were collected and allowed to become embryonated. They were then combined into two cultures and placed in an electric refrigerator where they remained for several months at a temperature of approximately 6°C. Both of these cultures, 303-326 and 315-344 days old, respectively, produced infection in chicks when passed through earthworms.

The writer has observed that heavily infested chickens kept in confinement may lose all or most of their capillaria infection within

a few months. In one recorded case, an experimentally infected New Hampshire chicken was used as a source of *C. caudinflata* cultures for 4 months. This bird had lost all its capillarids, however, by the time it was killed 10 months following its initial infection.

Experiments reported in this paper show that low temperatures which prevail in Northern Iowa during the winter months are undoubtedly fatal to many *C. caudinflata* eggs. Nevertheless, the test which follows shows that some worms of this species are able to overwinter in this region.

On May 12, 1942, two New Hampshire chickens, Nos. 7385 and 7448, about 13 weeks old, were each given 50 earthworms from a pen where chickens infected with *C. caudinflata* had been kept during the previous summer, no chickens having been in the pen since Oct. 22, 1941. On the following day each chick received 50 earthworms from this pen. Chicks Nos. 7408 and 7444, New Hampshire chicks of the same hatch, were given no earthworms and thus served as cage controls.

Each of the chickens receiving earthworms became infected with *C. caudinflata*. Five males and four females were recovered from No. 7448, and two females were obtained from No. 7385. Neither of the control chicks became infected.

This test shows that *C. caudinflata* survived a period of 202 days from October to May and was then transmitted to chickens the following spring by feeding infected earthworms. It is not known whether *C. caudinflata* survived through the winter in the embryonated egg stage, in the earthworm or in both.

EXPERIMENTS OF THE TRANSMISSION OF *C. caudinflata* BY SPECIES OF EARTHWORMS OTHER THAN *H. (A.) caliginosus*

Two attempts were made to transmit *C. caudinflata* by means of the manure worm *Helodrilus (Eisenia) foetidus*. On one occasion a chick received two earthworms of this species after they had been exposed to embryonated eggs of *C. caudinflata* for 35 days, but the chick did not become infected. In a second trial seven *H. (E.) foetidus* infected, by capillary pipette, with embryonated *C. caudinflata* eggs 26 days previously, were given to a chick. When this chick was killed 25 days later, no capillarids could be found. Several attempts to transmit *C. caudinflata* by using "night-crawlers," *Lumbricus terrestris*, as intermediate hosts gave negative results.

DISCUSSION

From the data obtained on the geographical distribution of *Capillaria caudinflata*, *C. columbae*, and *C. retusa*, it appears that *C. columbae* is the predominant species east of the Appalachian mountains whereas *C. caudinflata* predominates in the Midwest region. Only three birds infected with *C. columbae* (all from Minnesota) have been received from localities west of the Mississippi river, and records of *C. caudinflata*

in the Atlantic coast area are almost as scarce. This finding is in contrast to the opinion previously held by some investigators, that *C. columbae* is the predominant species throughout the United States.

One wonders that more records of capillarids were not found from states west of Iowa, Kansas being the only state listed. Since hundreds of chickens were received from this area, especially from the states east of the Rocky Mountains, it seems likely that capillarids may not be as abundant in this area as they are farther east. The absence of *C. caudinflata* from arid regions would be understandable, due to the necessity of using earthworms as intermediate hosts, but does not account for their apparent absence in regions having abundant moisture.

Eggs of *C. caudinflata* can be distinguished from those of *C. columbae* in several different ways. The difference in the length of the incubation period is one possible method, *C. columbae* requiring only 6–8 days whereas *C. caudinflata* requires 11–12 days. The shape of the eggs provides another distinguishing characteristic. Eggs of *C. caudinflata* are narrower in proportion to their length than those of *C. columbae*, and the latter frequently are more or less asymmetrical whereas the eggs of *C. caudinflata* are rarely so. Wehr (1939) found that the measurements of 25 eggs of *C. columbae* vary from 50 μ to 55 μ in length and from 27 μ to 31 μ in width. The measurements for a like number of *C. caudinflata* eggs, as recorded in this paper, were 50 μ to 59 μ long and 21 μ to 24 μ wide. A third difference is the character of the egg shells. The egg shells of *C. caudinflata* are truly punctate shells. When a microscope is focused on the surface of the shells and the light properly adjusted, pinpoint areas of brighter illumination may be seen dotting the surface. One can see the surface markings even more advantageously under dark-field illumination and especially so in embryonated or partially embryonated eggs. Eggs of *C. columbae* are also said to have punctate shells, but the writer has observed a different type of marking. To describe their surface as having an etched or sculptured appearance conveys a better picture than to speak of them merely as being punctate. As in the case of *C. caudinflata*, the surface markings appear to have no characteristic design or pattern. Since the eggs of *C. retusa* have not been studied, it is not known whether they possess any outstanding characteristics sufficiently constant to allow one to distinguish them from eggs of *C. columbae* or *C. caudinflata*.

The environmental factors influencing the development of a parasite egg are often of great significance so far as they relate to control measures. In the experiments conducted on the effect of temperature on *C. caudinflata* eggs, unembryonated eggs were able to withstand low temperature better than embryonated eggs, but the latter were better able to withstand high temperatures. This conclusion coincides with the results obtained by Wehr (1939) who stated that, at the same temperature, embryonated eggs of *C. columbae* are apparently viable for a shorter time than non-embryonated eggs. It should be noted that the temperature extremes to which these capillaria eggs were subjected, i.e.,

approximately 14°F. to 104°F., are within the temperature range of much of the northern half of the United States. It is therefore probable that many *C. caudinflata* eggs are actually killed during extreme weather conditions in this region.

Levine (1937) reported that embryonated eggs of *C. columbae* were dead 14 days after being dried by an electric fan. Wehr (1939) working with the same species stated that air drying partially embryonated eggs for 24 hours was lethal to the eggs. On the contrary, the writer has found that drying embryonated eggs of *C. caudinflata* for 24 hours served in some way as a stimulus for the hatching of the eggs when water was added to the culture.

Capillaria caudinflata is readily transmitted to chickens through earthworms of the species *Helodrilus (Allolobophora) caliginosus*. Carefully controlled experiments have shown that this species of earthworm serves as a true intermediate host. During these experiments, several thousand earthworms have been used. Obviously, it would be almost impossible to make a specific identification of each individual earthworm. However, specimens have been carefully identified from representative samples taken in the various places from which the worms were collected. Although it is possible that specimens of some other species may have been included in a few cases, the great majority of the worms are known to be *H. (A.) caliginosus*.

On March 27, 1942, some 5 months after the writer had first transmitted *C. caudinflata* with earthworms tentatively identified as *H. (A.) caliginosus*, specimens of these earthworms were sent to the Bureau of Animal Industry, U. S. D. A., Washington, D. C. These earthworms were identified as *H. (A.) caliginosus*, thus confirming the writer's identification.

Just as the discussion and conclusions of this investigation were being written, preparatory to its submission to the graduate faculty as a doctoral thesis, a note by R. W. Allen and E. E. Wehr (1942) entitled, "Earthworms as possible intermediate hosts of *Capillaria caudinflata* of the chicken and turkey," appeared in the Proceedings of the Helminthological Society of Washington. These authors apparently showed that *C. caudinflata* may be transmitted through earthworms to chickens and turkeys, although their experiments were not controlled and therefore failed to prove that the earthworms served as true intermediate hosts.

According to the note by Allen and Wehr, all results of their experiments were obtained subsequent to March 27, 1942. It appears that these investigators had no evidence of transmission until April 18, 1942, whereas experiments reported in this paper show that the writer had obtained evidence of transmission to chickens some 5 months before these investigators began their experiments. It should be further noted that the writer had transmitted *C. caudinflata* to turkeys about 2 weeks earlier than the date recorded by Allen and Wehr.

The utilization of earthworm digestive juice for causing *C. caudinflata* embryos to become motile and to hatch provides a valuable research

technique which was used on one occasion to determine maturity in embryos and once to determine mortality in eggs subjected to low temperature. It is unfortunate that this method was not developed earlier so that other experiments involving the effect of temperature on embryonated eggs could have been repeated.

Studies on the development of *C. caudinflata* larvae indicated that considerable development occurred in the earthworm, the larvae probably moulting at least once during this period. After the earthworms were eaten by a chicken, the oesophageal region of the larva developed rapidly, but there was little growth in the intestinal region for several days. By the twelfth day, however, the intestine had grown until it was approximately equal to the length of the oesophageal region. A moulting larva was found on the twelfth day in one chicken, together with sexually undifferentiated larvae and immature male and female worms. The secondary sexual organs such as the caudal alae of the male and the ovijector of the female were not yet present in 15-day specimens. The ovijector did not appear in females until the seventeenth day of development in the chicken, and the secondary sexual organs of the male did not appear until the nineteenth day.

Capillaria caudinflata is primarily a parasite of the duodenal loop of both chickens and turkeys. When the intestine was examined in five approximately equal parts, it was found that a little over half the worms were in the duodenal loop of the chicken and turkey and that about 90 per cent of them were in the upper two-fifths of the chicken intestine, while none were in the ceca of either the chicken or turkey. Therefore, references by earlier investigators to the presence of this species in the ceca of birds must be a case of mistaken identity of the species found in the ceca.

SUMMARY

1. The capillarid of the lower digestive tract of chickens and other avian hosts, formerly referred to as *Trichosoma longicolle* Rudolphi, 1819, or other questionable synonyms, is properly called *Capillaria caudinflata* (Molin, 1858).

2. In a geographical distribution study, *Capillaria caudinflata* was collected from 13 different states, *C. columbae* from 11 states, and *C. retusa* from 3 states. *C. caudinflata* was more prevalent than *C. columbae* in the Midwest, but the reverse was true in states east of the Appalachian Mountains.

3. *Capillaria caudinflata* could not be transmitted by feeding embryonated eggs directly to chickens.

4. Earthworms of the species *Helodrilus* (*Allolobophora*) *caliginosus* served as true intermediate hosts to *C. caudinflata*.

5. Attempts to transmit *C. caudinflata* by using earthworms of the species *Helodrilus* (*Eisenia*) *foetidus* and *Lumbricus terrestris* were unsuccessful.

6. The life cycle of *Capillaria caudinflata* required 42-45 days, 11-12 days of which were needed for embryonation of the ova, 9 days for devel-

opment in the earthworm, and 22-24 days for development in the chicken.

7. All attempts to utilize various species of grasshoppers, beetles, houseflies, ants, and sow-bugs as intermediate hosts were unsuccessful.

8. Eggs of *Capillaria caudinflata* can be distinguished from those of *Capillaria columbae* by differences in their size, shape, and the surface markings of their shells.

9. Temperatures corresponding to the extreme outdoor conditions in Northern Iowa were detrimental to *Capillaria caudinflata* eggs; however, this species was able to overwinter in poultry yards of Northern Iowa. Unembryonated eggs were able to withstand low temperature better than embryonated eggs but the latter were better able to withstand high temperature.

10. When *Capillaria caudinflata* eggs were treated *in vitro* with filtered digestive juice of earthworms they hatched within a few minutes, thus providing a valuable technique for proving or disproving the viability of the embryos.

11. The various developmental stages in the life cycle of *Capillaria caudinflata* have been studied.

12. *Capillaria caudinflata* was transmitted to a turkey and to three English sparrows, but a pigeon and a White Pekin duck failed to become infected after receiving infected earthworms.

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PLATE I

Capillaria caudinflata

- Fig. 1. Unsegmented egg showing translucent equatorial spot.
2. Two-cell egg at about 24 hours of development.
3. Three-cell egg at about 26 hours of development.
4. Four-cell egg. The division of the central cell to produce the fourth cell is atypical.
5, 6. Eggs after 45 hours of development. Approximately 8 blastomeres.
7. Egg after 70 hours incubation showing peripheral arrangement of the blastomeres.
8. Egg after 80 hours of development showing embryo shrunken away from the egg shell. The embryo is nonmotile at this stage.
9. At 100 hours the "bean-shaped" embryo shows a translucent end.
10. The embryo becomes S-shaped due to rapid elongation.
11, 12. Mature embryos photographed after 11 days of development.
13. First stage larvae escaping from the egg. \times ca 120.
14. Larva after 15 days of development in the earthworm. \times ca 245.
15. Larva after 5 days of development in the chicken. \times ca 65.
16. Moulting larva shown on the twelfth day of development in the chicken. \times ca 120.
17. Lateral view of the caudal end of a young male seen on the twelfth day of development in the chicken.
18. Lateral view showing the caudal end of the adult male.
19. Caudal end of adult male obtained from the English sparrow. Note the lateral alae and the heart-shaped bursa-like membrane.
20, 21. Lateral views of the caudal end of adult males from chickens.
22. Vulval region of adult female showing the ovijector.
23. View showing the vulval region in a 12-day immature female. No ovijector has yet been formed. \times ca 350.
24, 25, 26. Views showing difference in appearance of the ovijectors in adult female worms from chickens. Note the egg being discharged in Fig. 26.
27. View of the body wall which has been removed and flattened to expose the two bacillary bands characteristic of *C. caudinflata*.

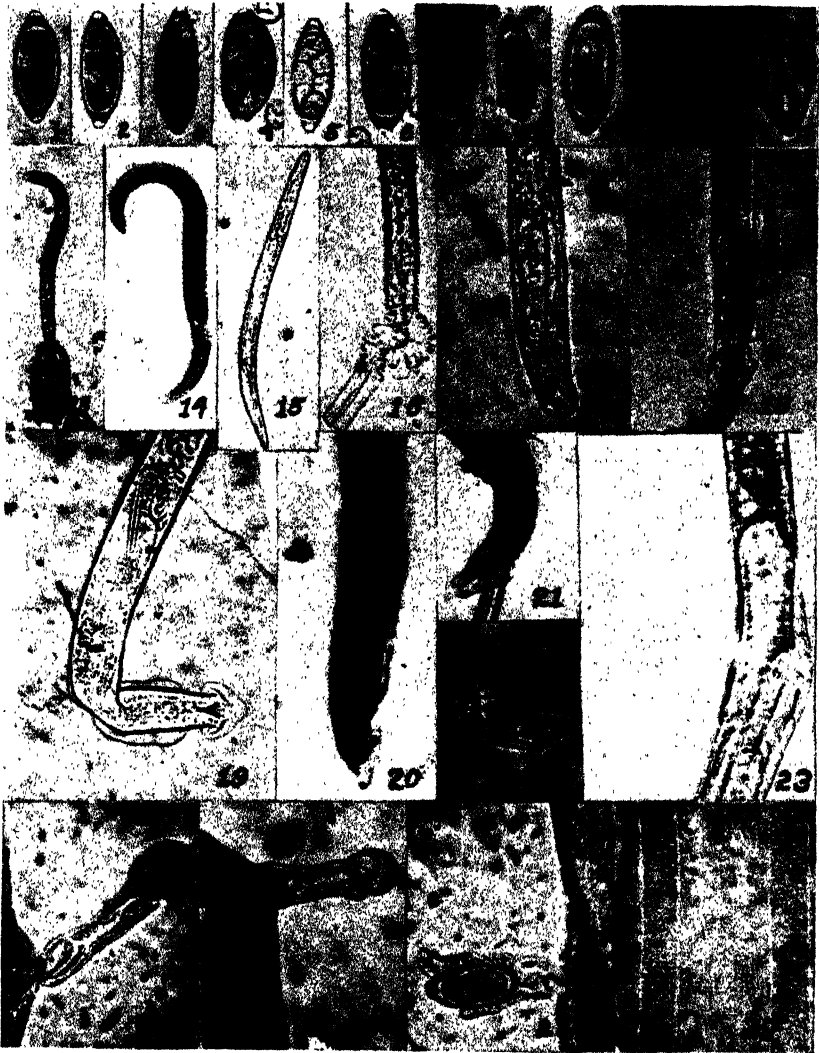


PLATE II

C. caudinflata larvae

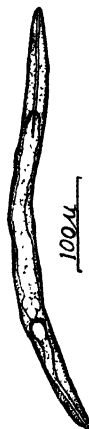
- Fig. 1. Recently hatched first-stage larva. Note the bilobed posterior end.
2. Larva after 15 days of development in the earthworm. This larva also has a bilobed posterior end.
3. Larva after 5 days of development in the chicken. Only part of the 5-day larvae have a bilobed posterior end.
4. Larva after 12 days of development in the chicken, showing oesophageal-intestinal junction. Note the two glandular cells located at this junction.
5. Larva after 9 days of development in the chicken.



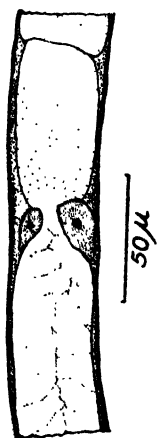
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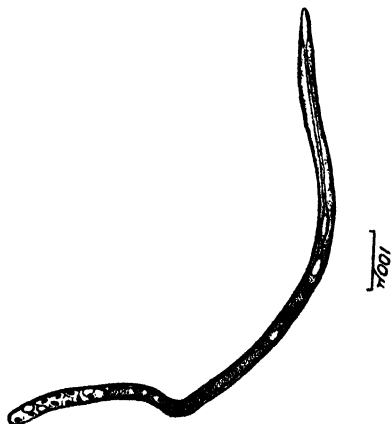
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5

EFFECT ON THE CHINCH BUG (*BLISSUS LEUCOPTERUS* SAY) OF CONTACT WITH VARIOUS DINITROPHENOLS AND OTHER DUSTS¹

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Three years ago Decker and Drake (1940) noted the high toxicity of dinitro-ortho-cresol toward the chinch bug, and suggested "a possible wide use for 3,5-dinitro-o-cresol . . . in temporary barrier construction." In May, 1942, W. P. Flint, State Entomologist of Illinois, distributed a mimeographed circular³ in which he very briefly stated his experiences, "for two full seasons," with dinitro-o-cresol (DN) dust as an acceptable chinch bug barrier.

Paucity of more definite information, and the danger of an impending shortage, during the present war, of creosote for chinch bug barriers made it desirable to investigate more thoroughly with laboratory tests the potentialities of some of the dinitrophenol dusts against the chinch bug. Investigations were begun in 1942 as soon as the bugs became numerous enough to be collected in the field in large numbers. The problem was continued until the species became too dispersed for practical collecting.

MATERIALS AND METHODS

All stages of the chinch bug, *Blissus leucopterus* Say, were field collected in various localities of western Iowa. Fresh young corn plants served as food for the captured stock colony. Overwintered adults from 1941, as well as first generation adults of 1942 were used. The data from these were kept separate. Results from the former are indicated by "Adult '41"; data from the latter are marked "Adult '42."

Some of the chemicals used were obtained in diluted mixtures from Standard Agricultural Chemicals, Inc., and from The Dow Chemical Company. Other compounds were secured in pure form from the above and other companies, and prepared as previously described by Tauber, Tauber, Joyce, and Bruce (1943).

The method used was essentially that already described by Tauber *et al.* (1943) in their contact toxicity tests with the dog tick, *Dermacentor variabilis*. Briefly stated, the technique consisted simply of setting up a 1-inch wide, circular barrier of dust, 6 inches in diameter, on a smooth paper surface. The 4-inch circle inside was dust free. Weighed amounts

¹ Journal Paper No. J-1119 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 372.

² Now with U. S. Army Sanitary Corps.

³ Not available in Library files. Received as a personal communication by C. J. Drake, Iowa State College.

of the dust were used in the construction of the barrier so that the amount distributed over the 1-inch band consisted of a sample equivalent to 65 to 75 pounds an acre. Insects to be tested were released in the dust-free 4-inch circle, and then recaptured after voluntarily crossing the level, 1-inch barrier of dust. Ten individuals were used in each trial; at least 10 trials, often as many as 40, were run in each test condition. Small camel-hair brushes were used to transfer or to guide the insects into vials in which they were retained during the period of observation. Vials were stoppered with cotton and kept at room temperature. One series of bugs was not fed after the tests; another series was given parts of fresh corn leaves daily, at least once and sometimes twice.

RESULTS AND DISCUSSION

Experiments of the first series in which the bugs were not fed after treatment were really of a preliminary, exploratory nature to determine the working range of dinitro-o-cresol. However, because the results nicely demonstrate the necessity of giving the chinch bug, especially the early instars, access to food when transferred to vials, tubes, or cages for laboratory study, these results on the unfed bugs are included here and presented in Table 1.

TABLE 1
EFFECT OF CONTACT WITH PYROPHYLLITE-DILUTED DINITRO-ORTHO-CRESOL
ON CHINCH BUGS UNFED AFTER TREATMENT

Stage	No. Tested	Concentration Percentage	Percentage Killed at				
			2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
Instar 1....	150	1.0	100				
Instar 2....	150	1.0	96*	96	97	100	
Instar 3....	150	1.0	24	27	36	62	82
Instar 4....	150	1.0	12	16	21	48	63
Instar 5....	100	1.0	8	16	32	71	96
Adult '41...	100	1.0	9	36	60	81	91
Instar 1....	150	2.0	100				
Instar 2....	150	2.0	100				
Instar 3....	150	2.0	92	96	97	99	100 in 30 hrs.
Instar 4....	100	2.0	56	66	72	82	100 in 45 hrs.
Instar 5....	200	2.0	68	76	86	90	98
Adult '41...	200	2.0	14	30	43	75	98
Instar 3....	200	4.0	100 in 1 hr.				
Instar 4....	200	4.0	100 in less than 2 hrs.				
Instar 5....	200	4.0	84	87	90	92	95
Adult '41...	200	4.0	24	76	90	92	94
Instar 3....	200	8.0	100 in less than 1 hr.				
Instar 4....	200	8.0	100 in less than 1 hr.				
Instar 5....	200	8.0	90	100 in 4 hrs.			
Adult '41...	200	8.0	50	94	96	100 in 20 hrs.	
Instar 3....	400	12.0	100 in less than 1 hr.				
Instar 4....	400	12.0	100 in less than 1 hr.				
Instar 5....	400	12.0	100 in less than 1 hr.				
Adult '41...	400	12.0	100 in less than 1 hr.				

* Throughout this paper results are expressed in nearest whole number.

Actually, tests with DN-o-C concentrations less than 1.0 per cent were made but showed no significant differences in mortality from the unfed controls, and these results are omitted from Table 1. In other words the effects of starvation and dehydration were so severe on unfed bugs that deprivation of food and water brought death before the toxic action of lesser DN-o-C concentrations had a chance to act. The mortality of unfed controls is given in Table 2.

TABLE 2
MORTALITY OF UNFED CONTROLS

Stage	No. Tested	Percentage Dead at				
		2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
Instar 1.....	160	8	14	30	59	80
Instar 2.....	170	3	5	12	54	79
Instar 3.....	200	1	4	8	35	73
Instar 4.....	220	1	2	4	14	42
Instar 5.....	240	1	2	3	12	25
Adult '41.....	200	1	3	4	12	29
Adult '42....	250	0	2	4	12	30

In most cases, the death rates shown in Table 2 are higher than those reported by Janes, Hager, and Carman (1935) for unfed chinch bugs held at various combinations of constant temperature and humidity. In every case, however, they also report that bugs having access to cotton moistened with water or sugar solution lived longer than starved controls. During the present series of experiments no attempt was made to regulate either temperature or humidity, and the fluctuating room conditions may have been more severe than the regulated environment used by Janes, Hager, and Carman (1935) with their chinch bugs.

In other tests to be described, except for some listed in Table 4, bugs had access to parts of young corn plants at all times. Sufficient trials were made to show that bugs had a higher survival on pieces of corn leaf rather than on sections of corn stalk. The results are in Table 3. Consequently, parts of corn leaves were placed in all vials with bugs of the subsequent tests.

Most mixtures with DN-o-C contained Pyrax or Pyrophyllite as diluents. These two materials are apparently trade names for similar

TABLE 3
MORTALITY OF CHINCH BUGS FEEDING ON STALKS OR LEAVES OF CORN PLANTS

Stage	No. Tested	Type of Food	Percentage Dead at				
			2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
Instar 5....	200	Stalks	1	3	5	13	17
Adult '42...	200	Stalks	1	3	5	10	15
Instar 5....	440	Leaves	0	1	2	5	9
Adult '42...	970	Leaves	0	3	4	8	11

substances. In some cases 320-mesh dusting sulfur was used as a diluent. Experiments showed that these diluting dusts, in themselves, had some toxicity toward the chinch bug, especially in the earlier instars. A summary of the data is found in Table 4.

According to the results of Table 4 there seems to be little choice among the types of sulfur; all had about the same effect on the chinch bug. Although the straight sulfur appears slightly less toxic than the

TABLE 4
MORTALITY OF CHINCH BUGS EXPOSED TO VARIOUS TYPES OF DILUENTS

Stage	No. Tested	Diluent	State of Nutrition	Percentage Dead at				
				2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
Instar 2....	150	Pyrophyllite	Unfed	8	48	63	99	100 at
Instar 3....	150	"	"	8	24	52	96	30 hrs.
Instar 4....	150	"	"	2	13	27	68	100 at
Instar 5....	150	"	"	0	1	5	15	36 hrs.
Adult '41..	250	"	"	4	22	34	47	98
Instar 5....	450	"	Fed	2	6	8	20	42
Adult '42..	450	"	"	2	5	8	18	51
Adult '42..	200	320-mesh sulfur*	"	1	2	4	18	29
Adult '42..	100	Conditioned sulfur	"	1	2	3	14	31
Adult '42..	100	Wettable sulfur	"	1	3	4	16	25
Adult '42..	100	Refined sulfur†	"	0	1	4	20	21
								24
								26

* Commercial dusting sulfur.

† Finely powdered sulfur as purchased from a drug store.

straight Pyrophyllite, when the former is combined with DN-o-C, the resultant mixture is more toxic than the same percentage of DN-o-C in Pyrophyllite. This difference is noted in Table 5.

Although circumstances of time, program of experiments, and availability of bugs made it impossible to compare 1941 overwintered adults with first-generation adults of 1942 throughout the investigations, the decreased vigor of aging overwintered forms makes it important that investigators on chinch bugs know the condition of their test insects.

One point in Table 5 which is perhaps deserving of additional investigation is the increase in toxicity resulting from an impregnation of the pyrophyllite with a solution of the DN-o-C. In our trials ether was used as the solvent. Weighed amounts of the DN-o-C were dissolved, and then mixed with the dry diluent. When larger volumes of the carrying solvent were necessary, a paste was formed, but this soon dried out and was easily reduced to a powder by screening and sieving. By impregnation, the toxic material is distributed over the diluent in molecular form rather than through the diluent in particulate form as when the dry ingredients are mixed. Better distribution through the diluent means increased chances of bringing the toxic agent in contact with the bug's body surface. Results in Table 5 indicate that impregnation was of special

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Adult '42...	970	Leaves	0	3	4	8	11

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Instar 3....	150	"	"	8	24	52	96	30 hrs.
Instar 4....	150	"	"	2	13	27	68	100 at
Instar 5....	150	"	"	0	1	5	15	36 hrs.
Adult '41..	250	"	"	4	22	34	47	98
Instar 5....	450	"	Fed	2	6	8	20	42
Adult '42..	450	"	"	2	5	8	18	51
Adult '42..	200	320-mesh sulfur*	"	1	2	4	18	29
Adult '42..	100	Conditioned sulfur	"	1	2	3	14	31
Adult '42..	100	Wettable sulfur	"	1	3	4	16	25
Adult '42..	100	Refined sulfur†	"	0	1	4	20	21
								24
								26

* Commercial dusting sulfur.

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(b) *S. lactis* cultures produced relatively large amounts of lactic acid but only very small amounts of acetylmethylcarbinol and diacetyl. Later, a rapid destruction of the carbinol and diacetyl occurred. Addition of citric acid did not appreciably increase the carbinol and diacetyl contents.

(c) Cultures of flavor organisms produced insignificant amounts of lactic acid. In unacidified milk small quantities of acetylmethylcarbinol and diacetyl were produced. Increasing the acidity by addition of lactic acid greatly increased the carbinol and diacetyl contents, the amounts of diacetyl being considerably larger than in butter cultures.

At 7°C.:

(a) Production of lactic acid, acetylmethylcarbinol, and diacetyl in butter cultures and in cultures of *S. lactis* and production of the carbinol and diacetyl in cultures of flavor organisms were much slower and the amounts were much lower than at 21°C.

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TABLE 5
MORTALITY OF FED CHINCH BUGS TO DINITRO-ORTHO-CRESOL DILUTED WITH
EITHER PYROPHYLLITE OR SULFUR

Stage	No. Tested	Conc. Percentage	Diluent	Percentage Dead at				
				2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
Instar 5 . . .	150	0.5	Pyrophyllite	2	6	12	21	33
Adult '42 . .	"	"	"	0	2	6	14	33
Instar 5 . . .	"	1.0	"	6	9	15	34	40
Adult '42 . .	"	"	"	14	18	28	44	50
Adult '42 . .	"	1.0	"	20	26	37	52	66
		by ether sol. impregnation	"					
Instar 5 . . .	200	2.0	"	2	5	13	30	45
Adult '42 . .	"	"	"	11	45	50	61	68
Adult '42 . .	"	2.0	"	28	49	58	68	79
		by ether sol. impregnation	"					
Instar 5 . . .	"	4.0	"	34	47	53	63	71
Adult '42 . .	"	"	"	51	79	82	90	92
Adult '42 . .	"	4.0	"	56	80	88	94	100
		by ether sol. impregnation	"					
Instar 5 . . .	250	8.0	"	91	93	93	94	94
Adult '42 . .	200	"	"	90	93	96	100 at 19 hrs.	
Instar 5 . . .	150	8.0	"	40	74	90	97	99
		with 5% oil	"					
Adult '42 . .	100	8.0	"	97	99	100
		by ether sol. impregnation	"					
Instar 5 . . .	150	12.0	"	82	83	85	93	95
Adult '42 . .	"	"	"	100				
Adult '42 . .	100	4.0	Sulfur	65	100 at 3 hrs.			
Adult '42 . .	"	8.0	"	95	100 at 2½ hrs.			
Adult '42 . .	300	12.0	"	96	100 at 2¼ hrs.			
Adult '42 . .	100	16.0	"	100 at ¾ hr.				

value in increasing toxicity of lower concentrations of the DN-o-C. At the 1.0, 2.0, and 4.0 per cent levels, these preliminary trials show a pronounced advantage from impregnation. However, the increase in toxicity becomes less evident with higher amounts of DN-o-C, and at the 8.0 per cent level only a slight increase in toxicity resulted from impregnation. Evidently at this level, and higher, the ratio of DN-o-C to diluent is sufficient to assure good distribution throughout the mixture when only mechanical mixing of dry ingredients is used. Manufacturers of dinitro mixtures state that solvent impregnation is entirely feasible without adding materially to the cost of preparation since the solvent could be recovered and used again.

Entomologists who have had experience in making dinitrophenol mixtures probably have also noticed that certain of these toxic principles diffuse quite readily even at room temperature. It is conceivable that even at lower concentrations this sublimation serves to equalize the distribution of the poison through the diluent. It is possible that mechanical mixes of low toxic content kept in tight containers at a warm tempera-

ture might eventually attain better distribution of the poison than that present just after mixing the dry materials. This possibility should be tested.

The higher mortality with sulfur mixes may be partially attributable to the increased adherence of the sulfur-DN-o-C combination to the chinch bug's body, as well as to the inherent toxicity of the sulfur itself. At least it seemed that the test bugs picked up more of the sulfur mixtures than the pyrophyllite dusts, after walking through the respective barriers.

TABLE 6
EFFECTS WITH DINITRO-ORTHO-SECONDARY-BUTYL-PHENOL ON CHINCH BUGS WITH
ACCESS TO FOOD AFTER TREATMENT

Stage	No. Tested	Conc. Percentage	Diluent	Percentage Dead at				
				2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
Instar 5....	250	1.0	Pyrophyllite	16	27	34	69	75
Adult '42..	"	"	"	13	26	41	78	81
Instar 5....	"	2.0	"	21	44	63	87	92
Adult '42..	"	"	"	30	53	70	92	96
Instar 5....	"	4.0	"	29	81	90	100 at 19 hrs.	
Adult '42..	"	"	"	37	79	99	100 at 19 hrs.	
Instar 5....	"	8.0	"	40	93	100 at 7 hrs.		
Adult '42..	"	"	"	45	98	100 at 6½ hrs.		
Instar 5....	"	8.0	"	47	72	83	95	100
Adult '42..	"	with 5% oil	"	53	79	87	100	

Even when special care was taken to prevent clumping during the preparation of barriers made with dusts containing 5 per cent oil, these mixtures tended to show a slight decrease in killing ability. (See Tables 6 and 7, also.) This lesser toxicity showed up in spite of a greater adherence by the oiled dusts. However, this property is likely in this case

TABLE 7
EFFECTS WITH DINITRO-ORTHO-CYCLOHEXYL-PHENOL ON CHINCH BUGS WITH
ACCESS TO FOOD AFTER TREATMENT

Stage	No. Tested	Conc. Percentage	Diluent	Percentage Dead at				
				2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
Instar 5....	250	1.0	Pyrophyllite	8	11	19	31	39
Adult '42..	"	"	"	7	13	21	38	46
Instar 5....	"	2.0	"	25	34	45	53	69
Adult '42..	"	"	"	21	47	54	60	73
Instar 5....	"	4.0	"	32	61	70	90	94
Adult '42..	"	"	"	38	64	76	96	98
Instar 5....	"	8.0	"	54	66	88	100	
Adult '42..	"	"	"	74	96	98	100 at 21 hrs.	
Instar 5....	"	8.0	"	60	84	91	95	100
Adult '42..	"	with 5% oil	"	47	75	88	98	98

TABLE 8

EFFECTS WITH AMMONIUM AND GUANIDINE DINITRO-ORTHO-CRESYLATES ON 1942 ADULT CHINCH BUGS WITH ACCESS TO FOOD AFTER TREATMENT

No. Tested	Chemical	Conc. Percentage	Diluent	Percentage Dead at				
				2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
100	NH ₄ DN-o-cresylate	8.0	Pyrophyllite	100
100	"	12.0	"	100 in 1 hr.
200	"	"	Sulfur	100 in 1½ hrs.
100	Guanidine DN-o-cresylate	8.0	"	24	54	65	90	96

to lead to a disadvantage in that the oiled dusts are less fluffy than dry dusts. Clumping tends to keep the oiled dusts in larger aggregates which are too heavy to stick to the insect's body. These oiled dusts were received, as such, from the manufacturers. Oil had been added in the belief that such treatment would make the dust stand up better against weathering. Decker (1943) reports in his field trials that the oiled dusts were "slightly more wind-resistant than those without oil." He also states that all dust lines, with or without oils, were subject to severe damage by heavy rains.

Laboratory tests were also made with four other dinitrophenols. Results are arranged in Tables 6, 7, and 8.

Examination of data in Tables 5, 6, and 7 indicates that the dinitro-o-secondary-butyl-phenol is more toxic than either dinitro-o-cresol or dinitro-o-cyclohexyl-phenol. The last named compound is generally slower than the other two in its lethal effects, especially at lower concentrations. These results agree with Decker's (1943) findings with a different procedure of tests of dinitrophenols with the chinch bug. If the preliminary results with ammonium dinitro-o-cresylate (Table 8) are indicative of data which may show up with further tests with larger samples of bugs, this compound shows even more promise. Decker's tests (1943) on foliage injury to corn and oats seedlings indicate that this ammonium salt is less injurious than either the dinitro-o-secondary-butyl-phenol or the dinitro-o-cresol, but more injurious than the dinitro-o-cyclohexyl-phenol. The ammonium compound has a disadvantage in being quite irritating to the respiratory tract of man, and greater precautions would be necessary in handling it. (Recent toxicological and pharmacological studies on effects of the sodium salt of 3-5-dinitro-o-cresol on mammals have been published by Ambrose, 1942.) At the 12 per cent level, substitution of sulfur for pyrophyllite as the diluent of the ammonium salt resulted in no significant difference. Death came so rapidly (1 to 1½ hours) that no attempt was made to analyze any difference in speed of action.

Theoretically, from what is now known of the toxicity of dinitrophenols, there is no apparent reason why the ammonium dinitro-o-cresylate should be more toxic than the straight DN-o-C. However, the ammonium salt is much more water soluble, and this property may increase

its ability to enter, dissolve in, and be distributed in the insect's interior which is bathed by a medium which is basically aqueous. It is hoped that this suggestion may be tested with experimental procedures in the near future.

Table 9 includes data from a variety of compounds furnished by different manufacturers for purposes of tests. Included are some additional dinitrophenol derivatives. None of the materials of Table 9 showed any striking toxicity toward the chinch bug, even when used, in some cases, as the pure compound. It should be stated, however, that certain of the preparations really do not have the physical properties necessary for use as a dust, and our tests, therefore, may not have afforded a fair measure

TABLE 9
EFFECTS WITH MISCELLANEOUS COMPOUNDS ON 1942 ADULT CHINCH BUGS WITH
ACCESS TO FOOD AFTER TREATMENT

No. Tested	Chemical	Conc. Percent- ages	Diluent	Percentage Dead at				
				2 hrs.	5 hrs.	10 hrs.	24 hrs.	48 hrs.
200	"Thanite"*	6.0	?†	2	3	4	8	11
100	Orthophenyl phenol	20.0	Loom Kill Talc	7	15	23	42	50
100	"	30.0	"	10	19	28	55	65
150	Di-(4-chlorophenoxy) methane	5.0	"	13	34	36	41	42
150	B-(4-tert.-butyl phenoxy) ethanol	5.0	"	13	20	21	22	30
150	2(2(2,4,5,6-tetra- chlorophenoxy)- ethoxy)ethyl chloride	5.0	"	4	7	10	15	18
150	x-chloro- phenothioxine	5.0	"	6	11	16	24	24
150	Plasticizer Chlor W*	5.0	?†	7	20	21	26	44
150	Phenothioxine	5.0	Loom Kill Talc	3	6	8	14	15
200	Tetra-chlorophenol	100.0	6	15	22	30	42
100	Penta-chlorophenol	100.0	25	72	87	98	99
100	Hexa-chlorophenol	100.0	16	50	70	88	90
150	p-nitro-phenotole	100.0	0	5	9	20	40
100	2-4 Dinitroso- resorcinol	100.0	2	8	10	50	19
100	3-5 Na salt of DN-o-cresol	100.0	38	82	87	100
150	2-4 Dinitro- chlorobenzene	100.0	4	7	11	23	52
100	Dicyclohexylamine salt of DN-o-cyclo- hexyl-phenol	20.0	?†	2	8	23	50	81
100	Ca-DN-o-cyclohexyl- phenate	100.0	2	9	21	38	54
100	DN-o-cyclohexyl- phenol benzoate	100.0	17	34	50	98	100
100	Na salt of DN-o- cyclohexyl-phenol	100.0	17	34	50	98	100

* These were commercial products whose composition was not available at this writing.

† These compounds were diluted when received from the manufacturer, and the diluent was not listed.

of their toxicity. Some were too sticky for dusting; others were too granular or too spicular and could not be reduced to fine enough particles by ordinary mortar and pestle.

CRITIQUE OF METHOD

During the course of this investigation it soon became apparent, from discussions with practical economic entomologists who might need to use toxic dust barriers for chinch bug control, that there were possible two quite divergent but equally reasonable techniques for making laboratory tests of dusts before field trials were inaugurated. These viewpoints on the manner of testing may be summarized as follows:

1. The test should be set up to give the bugs a maximum charge of the toxic agent by actually shaking them in the dust until they are equally and thoroughly covered, or

2. The technique should be established on the premise that all bugs traversing a barrier will not necessarily receive equal contact with the toxic agent, and thus set up a test in which the bugs voluntarily walk over the barrier and pick up varying amounts of the dust.

Plausible arguments may be brought forward for either idea. The first viewpoint is based primarily on the suggestion that with a properly constructed dust barrier all bugs that attempt to cross will lose their footing, tumble and roll, and become thoroughly coated with the dust. This assumption is basically correct, but there is some question as to how long even a properly constructed barrier will remain in perfect condition. Wind may scatter and flatten the crest of the barrier so that some bugs can get across without wallowing in the dust. Straws, leaves, and other litter blown across a barrier will also tend to drag down and level off a barrier. Barrier dust may settle, become more compact, and be less likely to offer a loose fluffy surface over which the bug must travel. Ground-moisture diffusing up through the barrier, or dew on the surface may make the dust clump together. Subsequent drying of the dampened dust may cause it to crust over. Even light rains may pack down the dust. Heavier rains will wash away and disperse the barrier. In short, it is conceivable that a barrier may not always be in ideal condition, though still serviceable if its toxic agents were originally present in sufficiently high concentration. If barriers would stay in perfect shape, their effectiveness could be retained with dusts containing lower concentrations of toxic substances. Costs of construction and maintenance could thus be kept down. However, since dust barriers are susceptible to such types of damage as enumerated above, their concentration of poisonous chemicals must be started high enough to keep the control measure effective in spite of damage, until repairs may be made. This is simply a reasonable margin of safety. A concentration which may be excessive under ideal conditions may be reduced to the minimum of effectiveness in a damaged or altered barrier. This concept is the foundation of the second viewpoint.

When barriers are altered from their original condition, it is possible

that many bugs will pick up only a trace of the dust, but that trace must be sufficient to kill. The second technique will then include results from some bugs which may have passed through the laboratory test barrier on their "toes", so to speak, and also from some bugs which may have been stampeded to tumble, wallow, and thus become thoroughly coated. This variability in dosage is an admitted fault of the technique. To kill those bugs which get through the barrier with the minimum of contact, somewhat higher concentrations of poison must be resorted to. If results from the second technique are depended upon as a lead for field trials, higher costs of toxic chemicals are implied.

With the first technique of shaking the bugs in the dust, equal dosages are assured, average killing time is decreased, and results are more consistent. With lower concentrations of toxic agent, 100 per cent kill can be quickly attained. With the second technique of allowing the bugs to walk through the dust, higher concentrations must be resorted to in order to kill all bugs within a time interval as short as that attained by thorough dusting with lower percentages of toxic dusts.

From one standpoint the two techniques differ in their intentions, and data from the two measurements should not be compared. The "thorough dusting" technique is essentially a method for concisely comparing the toxicity of several compounds. The "walking through" technique is essentially a test of method, namely: can chinch bugs be killed by using a dust barrier containing a certain chemical. Actually, the question of an effective concentration for practical field usage is not definitely settled by either method. Vagaries of wind, temperature, humidity, ground-moisture, etc. can hardly be duplicated with laboratory methods. Neither technique takes into account such complications as that involved in the chinch bug's habit of crawling under clods or brushing against foliage, thereby rubbing off some of the poisoned material which it may have picked up in crossing a dust barrier.

SUMMARY AND CONCLUSIONS

1. By employing a simple flat dust barrier, 1 inch wide, on a smooth surface, in concentrations equivalent to about 70 pounds an acre, tests were made on the effect of contact by the chinch bug (*Blissus leucopterus* Say) with various dinitrophenols and other dusts.

2. Under laboratory conditions of uncontrolled temperature and humidity, chinch bugs, especially the younger instars, are considerably debilitated by dehydration and starvation. Bugs so weakened are much more susceptible to contact with dinitro-o-cresol than those with access to food after exposure to the dust.

3. When dinitro-o-cresol is diluted with 320-mesh sulfur, the mixture is more toxic than that of equal DN-o-C concentration with Pyrax or Pyrophyllite as the diluent. Sulfur mixes seemed to adhere better to the body of the insect.

4. Up to about 8 per cent DN-o-C, impregnation of the diluent with the DN-o-C in liquid form increases the toxicity of the resultant mixture

over that of the same concentration freshly mixed with ingredients in a dry state. Putting the toxic agent into solution simply gives a chance for more thorough molecular dispersal throughout the diluent. Impregnation is perhaps of value only in mixing dusts with a low DN-o-C content. With higher concentrations mechanical mixing of dry ingredients can be sufficient. With some dinitro compounds sublimation of the poison may contribute to the dispersal of the toxic agent through the dry diluent.

5. Unless special care is taken with dinitrophenol dusts containing oil, the oiled mixtures will tend to clump into aggregates which are too heavy to lodge securely on the chinch bug's body surface. The oil does make the mixture somewhat "sticky," and with proper precautions in the preparation and use of a fresh, fluffy barrier, the addition of oil increases adherence of the dust. When clumping occurs, this advantage is largely lost. This point might be especially true under field conditions when larger aggregates of the oiled dust would be more easily dislodged by brushing against clods, plant growth, etc.

6. Dinitro-o-secondary-butyl-phenol was the most toxic of the dusts tested with large samples of bugs. A 4 per cent mixture in Pyrophyllite killed all samples of 1942 adults within 19 hours.

7. Dinitro-o-cresol and dinitro-o-cyclohexyl-phenol showed up about equally well at some levels of concentration. Eight per cent of the former in Pyrophyllite killed 100 per cent 1942 adults in 19 hours; 8 per cent of the latter killed all within 21 hours. Dinitro-o-cyclohexyl-phenol is slower in beginning to show its lethal properties, especially at lower concentrations.

8. Preliminary tests on small samples seemed to indicate that the ammonium dinitro-o-cresylate is extremely toxic to the chinch bug. Eight per cent in Pyrophyllite produced 100 per cent mortality of 1942 adults within 2 hours. This compound was received too late last season to make extended tests.

9. A critique of method is offered.

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IMPORTANT FACTORS IN FLAVOR DEVELOPMENT IN BUTTER CULTURES¹

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The compounds produced during the ripening of butter cultures and the factors influencing their formation have been extensively investigated (1). It is generally recognized that the usual butter cultures contain two types of bacteria, one of which (*Streptococcus lactis*) produces primarily lactic acid and often small amounts of acetylmethylcarbinol, diacetyl, and volatile acids, while the other (flavor organisms of the *Streptococcus citrovorus* and *Streptococcus paracitrovorus* group), under favorable pH conditions, produces relatively large amounts of acetylmethylcarbinol, diacetyl, and volatile acids from citric acid. In butter cultures the lactic acid produced by *S. lactis* decreases the pH to the point where acetylmethylcarbinol and diacetyl accumulate in significant amounts through the activity of the flavor organisms.

There have been certain suggestions that the commonly accepted relationships in the production of flavor in butter cultures can be modified in one way or another. Because of these, trials were conducted for the purpose of showing the importance of certain factors in the formation of various compounds in butter cultures. Representative results from certain of the trials are given in graphs.

METHODS

SOURCES OF CULTURES

The butter cultures used were from the collection maintained at the Iowa Agricultural Experiment Station. The cultures of *S. lactis* and of flavor organisms were recent isolations from the butter cultures.

PREPARATION OF MILK CULTURES ANALYZED

Whole milk was pasteurized in a flask by heating in boiling water for 1 hour and then cooling to 21°C. The milk was inoculated with the desired culture (in milk) and thoroughly mixed by shaking. In the usual procedure one-half the contents of the flask was then transferred, in portions of 200 ml. each, to sterile pint milk bottles. Either citric or lactic acid was added to the remainder of the milk and thoroughly distributed, after which 200 ml. portions were transferred to bottles; citric acid was added as a sterile aqueous solution (50 g. per 100 ml. water), and lactic acid was added directly from the original container (about 85 per cent). One-half of the bottles in each series were cooled to 7°C. in ice

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water and incubated at that temperature, while the others were incubated at 21°C. Bottles from each temperature were examined at intervals for lactic acid, acetylmethylcarbinol, and diacetyl. Exceptions to the general procedure were that in some trials with *S. lactis*, citric acid was not used, and in the trials with flavor organisms 21° or 7°C. was used for incubation instead of both temperatures.

The amount of citric acid added regularly was 0.15 per cent (of the crystallized compound) while the amount of lactic acid varied somewhat but gave the original milk an acidity from 0.45 to 0.76 per cent acid.

ANALYTICAL PROCEDURES

Diacetyl was determined on a 50 or 100 g. sample by the colorimetric method of Prill and Hammer (4), with minor modifications used by Hoecker and Hammer (2). Acetylmethylcarbinol was determined colorimetrically on a 20 or 50 g. sample using the procedure described (2) for its estimation in butter. In determining lactic acid an 18 g. sample was titrated with 0.1N sodium hydroxide, using phenolphthalein as the indicator, and the results calculated as lactic acid.

EXPERIMENTAL

PRODUCTION OF VARIOUS COMPOUNDS BY BUTTER CULTURES

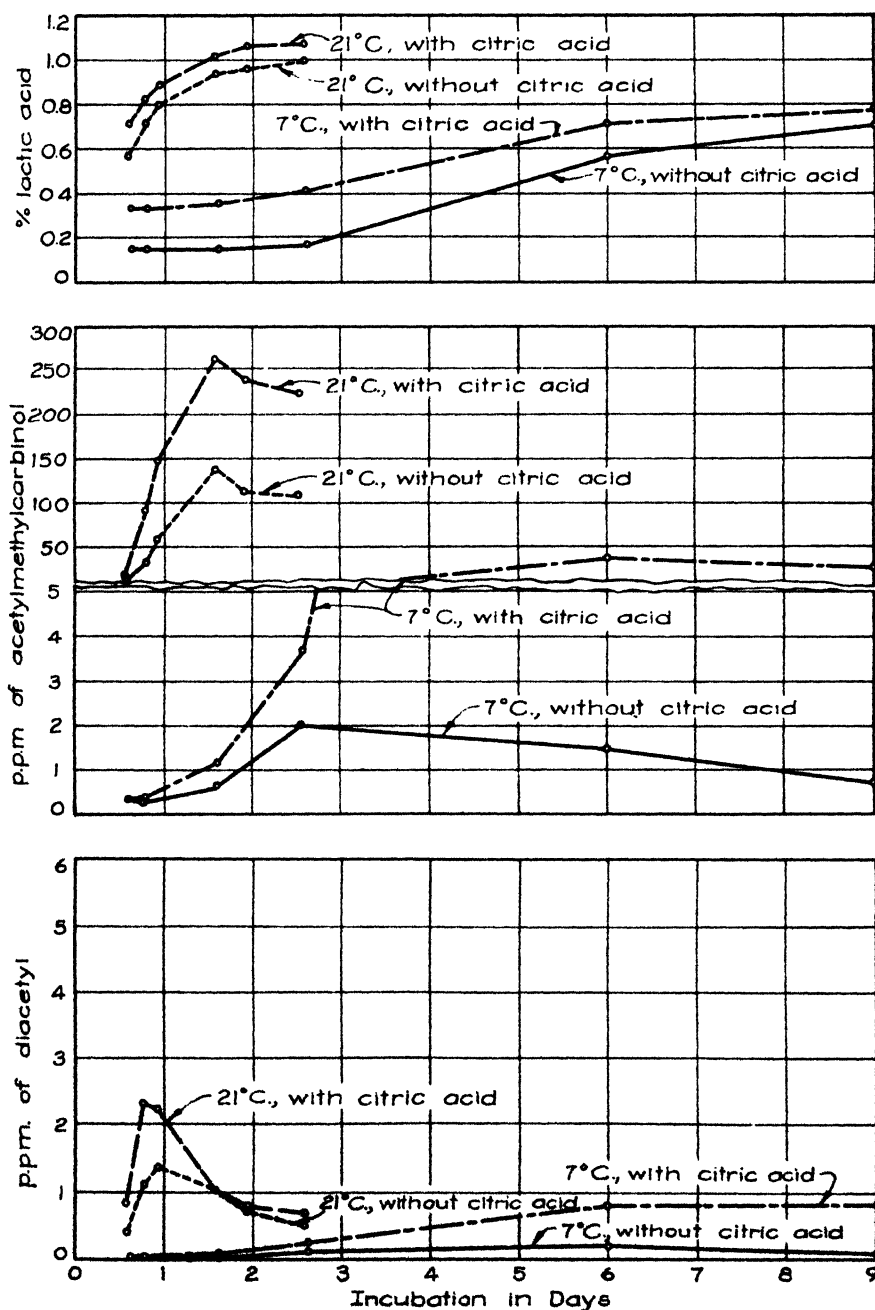
Production of lactic acid, acetylmethylcarbinol, and diacetyl in butter cultures was studied in two trials, using 0.1 per cent inoculation. Data obtained in one of the trials are given in graph 1.

LACTIC ACID. At 21°C. lactic acid was produced rather rapidly until the acidity reached about 0.80 per cent after which production was slower. The maximum acidity was noted at the final determination, which was made after 62 hours, and was 1.00 per cent. At 7°C. there was little increase in acidity after 63 hours, but after 9 days the acidity had reached 0.70 per cent.

Addition of citric acid did not affect the rate of acid production at either temperature but slightly increased the total acidities. The maximum values noted with citric acid added to the milk were 1.07 per cent at 21°C. and 0.78 per cent at 7°C.

ACETYLMETHYLCARBINOL. At 21°C. production of the carbinol was relatively slow for a time, but as the acidity increased carbinol production also increased; a maximum of 138.5 p.p.m. was noted after 38 hours, when the acidity was 0.94 per cent. Later, there was a small decrease in the carbinol. At 7°C. a small but significant amount of acetylmethylcarbinol was produced, the maximum value noted being 2.0 p.p.m. after 63 hours; later, there was a decrease in the amount.

With citric acid added to the milk there was a large increase in the carbinol production at both temperatures, the maximum amounts noted being 263.8 p.p.m. after 38 hours at 21°C. and 41.3 p.p.m. after 6 days at 7°C. At both temperatures production of considerable carbinol was followed by a decrease in the amount.



Graph 1. Production of various compounds by butter cultures at 21° and 7°C., with and without citric acid added to the original milk.

DIACETYL. At 21°C. diacetyl was produced rather rapidly, the maximum amount noted being 1.37 p.p.m. after 22 hours. The amount then decreased rather rapidly; the decrease was more rapid on a percentage basis than that with acetylmethylcarbinol, and after 62 hours the diacetyl content was 0.51 p.p.m. At 7°C. production of diacetyl was relatively slow, the maximum amount noted being 0.20 p.p.m. after 6 days; some destruction of diacetyl was noted after 9 days.

When citric acid was added to the milk the diacetyl production at 21°C. was greatly increased; after 19 hours 2.32 p.p.m. was present. There then was a rapid decrease until after 62 hours the diacetyl content was 0.68 p.p.m. At 7°C. citric acid also increased the production of diacetyl; after 9 days the content was 0.82 p.p.m., which was the maximum value noted.

PRODUCTION OF VARIOUS COMPOUNDS BY *S. lactis*

Production of the various compounds in milk cultures of *S. lactis* was studied in six trials. In two trials 0.1 per cent inoculation was used and citric acid was added to part of the milk, while in four trials 0.9 per cent inoculation was used and no citric acid was added. Graph 2 is based on the data obtained in one of the trials.

LACTIC ACID. Production of lactic acid by *S. lactis* was very similar to its production by butter cultures. At 21°C. the acidity increased rather rapidly for a time and then much more slowly. The maximum acidity noted was 0.94 per cent after 46 hours and also after 62 hours. At 7°C. very little acid was produced early in the incubation, but after 9 days 0.78 per cent acid was present.

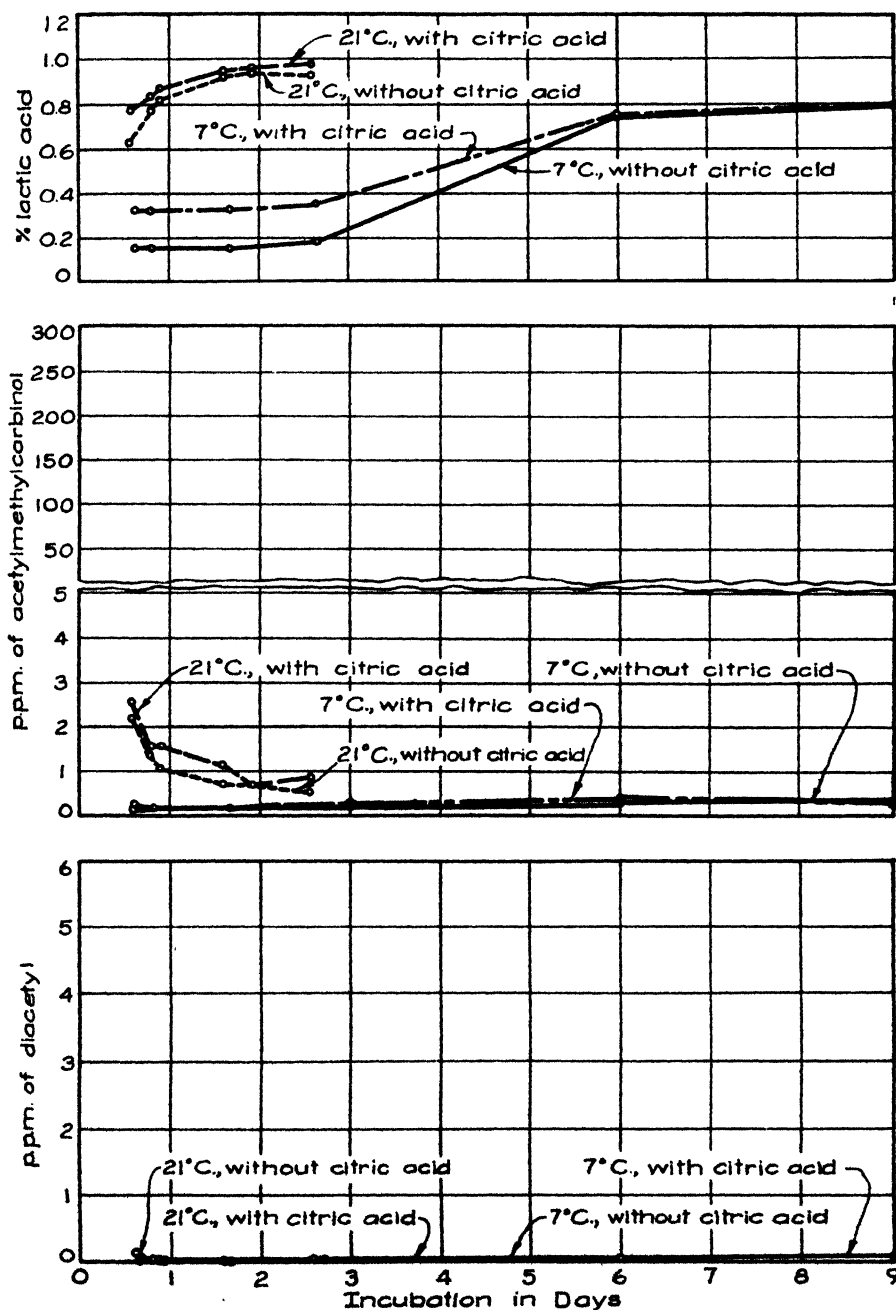
Addition of citric acid slightly increased the total acidity at both 21° and 7°C., the maximum values being 0.98 per cent after 62 hours at 21°C. and 0.80 per cent after 9 days at 7°C.

ACETYLMETHYLCARBINOL. At 21°C. a small amount of acetylmethylcarbinol was produced. After 14 hours 2.18 p.p.m. was noted after which there was a fairly rapid destruction until only 0.53 p.p.m. remained after 62 hours. In other trials the maximum values on carbinol were obtained after longer incubation (24 to 31 hours). At 7°C. acetylmethylcarbinol production during the 9-day incubation was insignificant, the maximum value noted being 0.29 p.p.m.

When citric acid was added, production of acetylmethylcarbinol was not significantly increased at either 21° or 7°C.

DIACETYL. At 21°C. diacetyl was produced in only very small amounts. The maximum value noted was 0.14 p.p.m. after 14 hours, and this had decreased to 0.05 p.p.m. after 19 hours. At 7°C. only traces of diacetyl were found.

With addition of citric acid there was no significant increase in diacetyl production.



Graph 2. Production of various compounds by *S. lactis* at 21° and 7°C., with and without citric acid added to the original milk.

PRODUCTION OF VARIOUS COMPOUNDS BY FLAVOR ORGANISMS

Production of the various compounds in milk cultures of flavor organisms was investigated in six trials using 0.5 per cent inoculation. In two trials 21°C. was used for incubation and in four trials 7°C. was employed. One-half the bottles in each trial had lactic acid added to lower the pH. Data obtained in one trial at 21°C. and in one trial at 7°C. are given in graph 3.

LACTIC ACID. At either 21° or 7°C. there was no significant change in acidity, either with or without addition of lactic acid to the original culture.

ACETYLMETHYLCARBINOL. At 21°C. acetylmethylcarbinol was produced in small amounts, the maximum value noted being 3.9 p.p.m. after 12 hours; there then was a rapid destruction until only 0.7 p.p.m. remained after 50 hours. At 7°C. some carbinol was produced although it was formed at a much slower rate than at 21°C.; the maximum value obtained was 2.56 p.p.m. after 7 days. No subsequent decrease was noted.

Addition of lactic acid to lower the pH greatly increased production of the carbinol. The maximum value noted at 21°C. was 111.5 p.p.m. after 50 hours, while at 7°C. it was 10.64 p.p.m. after 7 days. No decrease was noted at either temperature.

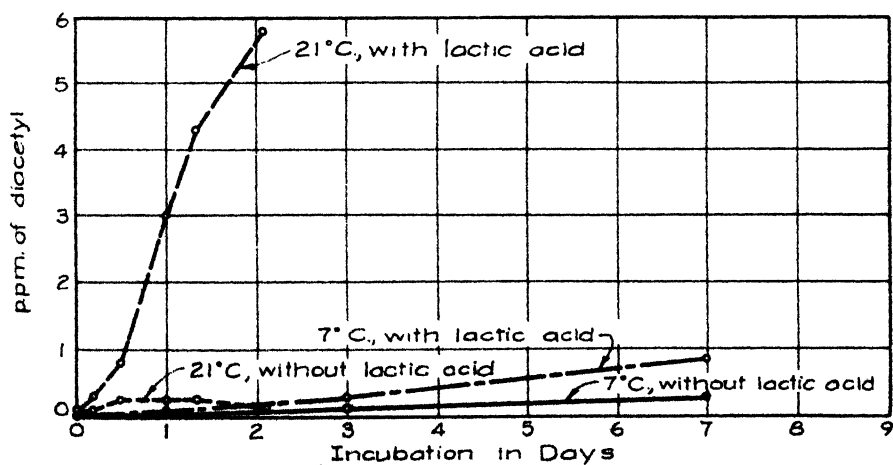
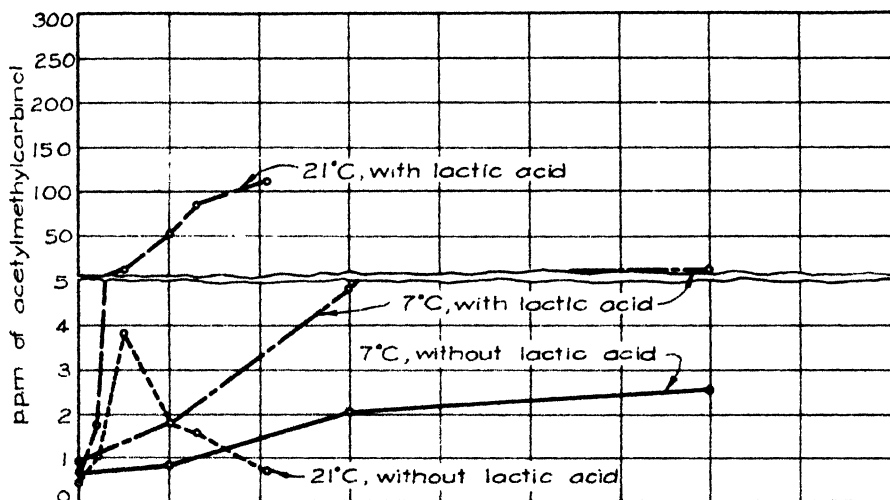
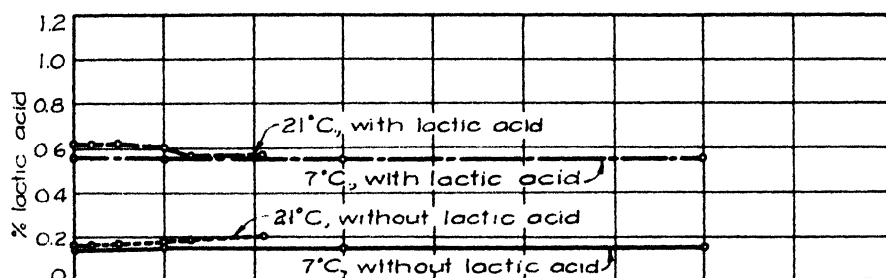
DIACETYL. At 21°C. a small amount of diacetyl was produced, the maximum value obtained being 0.28 p.p.m. after 32 hours. During the latter part of the incubation a decrease occurred. At 7°C. the production of diacetyl was slower than at 21°C., the maximum amount noted being 0.24 p.p.m. after 7 days when the final determination was made.

When lactic acid was added to lower the pH, production of diacetyl was increased, especially at 21°C. where a value of 5.78 p.p.m. was obtained after 50 hours. At 7°C. the maximum value obtained was 0.85 p.p.m. after 7 days. No decrease was noted at either temperature.

DISCUSSION

The increased amounts of acetylmethylcarbinol and diacetyl in butter cultures after considerable lactic acid had been formed emphasize the importance of pH in the accumulation of these compounds. The decreases from the maximum carbinol and diacetyl contents presumably were due to reduction of these compounds to 2,3-butylene glycol by the butter culture organisms. The large increases in the carbinol and diacetyl resulting from addition of citric acid show the importance of this acid as a source of flavor in butter cultures.

The similarity in the lactic acid production in cultures of *S. lactis* and in butter cultures and the small amounts of acetylmethylcarbinol and diacetyl produced by *S. lactis* indicate that the main function of this species in butter cultures is production of lactic acid. With the *S. lactis* strains studied, the amounts of acetylmethylcarbinol and diacetyl produced were not significant; apparently, other strains of *S. lactis* or other



Graph 3. Production of various compounds by flavor organisms at 21° and 7°C., with and without lactic acid added to the original milk.

species of lactose-fermenting streptococci may produce more carbinol and diacetyl (1). Wiley, Cox, and Whitehead (6) reported working with cultures of *Streptococcus cremoris* which produced as high as 1.0 p.p.m. diacetyl in milk. Other investigators have isolated different species of lactose-fermenting streptococci which produce relatively large amounts of lactic acid, acetylmethylcarbinol, and diacetyl (2). Addition of citric acid to the *S. lactis* cultures did not significantly change the products formed.

The chief function of the flavor organisms in butter cultures is the production of flavor compounds from citric acid. Due to the unfavorable pH, production of acetylmethylcarbinol and diacetyl was much less in unacidified milk than in milk to which lactic acid was added, and the destruction of these compounds was more rapid. In the acidified cultures the carbinol contents were very similar to those of butter cultures, whereas the diacetyl contents were somewhat higher. Citric acid was not added to the cultures, but addition of this acid has been shown to greatly increase production of carbinol and diacetyl by the flavor organisms (3). Although the strains of flavor organisms studied showed no significant change in acidity, certain strains of *S. paracitrovorus* may be much more active acid producers.

While acetylmethylcarbinol and diacetyl contents may rapidly decrease in butter cultures or in pure cultures of the butter culture organisms, the data indicate that a decrease in lactic acid does not occur, even with relatively long incubation periods.

Although production of the various compounds was more rapid at 21° than at 7°C., the general fermentation brought about by butter culture organisms was the same at the two temperatures. With long incubation periods at 7°C. considerable amounts of lactic acid were formed by *S. lactis*, while small but significant amounts of carbinol and diacetyl were formed by the flavor organisms. Even in the unacidified cultures of the flavor organisms the amounts of carbinol and diacetyl compared favorably with those produced by butter cultures and were considerably higher than those produced by *S. lactis*. Wiley, Cox, and Whitehead (5,6) suggested that in butter cultures incubated at 21°C., where acid production is rapid, the flavor organisms are mainly responsible for the formation of acetylmethylcarbinol and diacetyl, while in butter cultures incubated at 7°C., where only small amounts of acid are produced, *S. cremoris* is mainly responsible for the formation of the compounds.

SUMMARY

At 21°C.:

(a) Butter cultures produced relatively large amounts of lactic acid, acetylmethylcarbinol, and diacetyl. At first, production of lactic acid was rapid while production of the carbinol and diacetyl was slow. As the acidity increased there was a more rapid production of the carbinol and diacetyl; later, a destruction of these compounds occurred. Addition of citric acid greatly increased the carbinol and diacetyl contents.

(b) *S. lactis* cultures produced relatively large amounts of lactic acid but only very small amounts of acetylmethylcarbinol and diacetyl. Later, a rapid destruction of the carbinol and diacetyl occurred. Addition of citric acid did not appreciably increase the carbinol and diacetyl contents.

(c) Cultures of flavor organisms produced insignificant amounts of lactic acid. In unacidified milk small quantities of acetylmethylcarbinol and diacetyl were produced. Increasing the acidity by addition of lactic acid greatly increased the carbinol and diacetyl contents, the amounts of diacetyl being considerably larger than in butter cultures.

At 7°C.:

(a) Production of lactic acid, acetylmethylcarbinol, and diacetyl in butter cultures and in cultures of *S. lactis* and production of the carbinol and diacetyl in cultures of flavor organisms were much slower and the amounts were much lower than at 21°C.

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A LIST OF IOWA ANTS¹

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Since the publication of the author's preliminary list of Iowa ants (1941a), extensive collecting in many parts of Iowa has made possible a more complete list, comprising 97 forms. Five of these forms are new to science.

The writer believes that the listed forms comprise a large percentage of the ants which exist in Iowa. Ten or perhaps even 20 additional species might be collected. To do so, however, would take years of intensive collecting and a systematic survey of every county. No such survey was possible for the writer. Instead, an attempt was made to collect in all the different ecological areas, and to find as many good collecting areas as possible. In the search for the latter the numerous state parks of Iowa proved very helpful. Backbone State Park in Delaware County deserves particular mention. At this park a new and extraordinary species, *Lasius* (A.) *plumopilosus* Buren, was found, along with several other species rare or lacking in other parts of the state.

In general there are only two main faunal areas in Iowa. The first and by far the largest may be termed the Mississippi area. It occupies the large portion of Iowa within the Mississippi River drainage system. It is characterized by an ant fauna much like that of the states farther east. The genera richest in species are *Formica* and *Lasius*, and to a lesser extent *Camponotus*, *Leptothorax*, *Aphaenogaster*, and *Myrmica*.

The second area is much smaller, comprising only the bluffs along the Missouri River. These bluffs consist of loess soil and are very steep and quickly drained, ecologically simulating the arid southwestern states or the Great Plains. This condition is reflected in the ant fauna. *Eciton* and *Iridomyrmex*, two genera found in these bluffs, are not represented in the rest of the state. Also found in this region are *Pheidole sitarches* Wheeler, *Paratrechina* (N.) *arenivaga* Wheeler, *Dorymyrmex pyramicus* (Roger), *Crematogaster minutissima missouriensis* Emery, *Camponotus caryae rasilis* Wheeler, and *Formica pallidefulva dolosa* Wheeler, species not found in the Mississippi area.

The area drained by the rivers and streams which flow into the Missouri River seems transitional between the Mississippi River drainage

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area and the Missouri River bluff area, and contains elements of both faunas.

At least two species, *Aphaenogaster treatae* Forel and *Ponera trigona opacior* Forel, have a discontinuous distribution, being found only in the bluffs of the Mississippi and Missouri rivers. These are southern species which appear to have crept northward only along the large rivers.

Two species, *Pogonomyrmex occidentalis* (Cresson) and *Formica fusca neoclara* Emery, were found in the Iowa State College collection labeled Sioux City, Iowa. Since both these species have heretofore been known only from the Great Plains and Rocky Mountains, their existence in Iowa should remain in doubt until validated. If these two species do occur in Iowa, they belong to the Missouri River bluff fauna.

In the following list, the writer has recognized only one infraspecific variant, the subspecies, in contradistinction to most of the older, and many of the recent, authors who recognize both subspecies and varieties. For as Creighton has shown (1938), there is no valid difference between the subspecies and the variety, and the retention of both ranks only complicates the nomenclature. The author has, therefore, raised all forms in this list formerly considered as varieties to subspecific rank. In some instances the raising of certain varieties to subspecific rank seems to cast doubt on their validity, and the author has discussed their taxonomic status.

Creighton states (1938) that all variants are probably geographical races. It seems likely, however, that many variants are ecological races rather than geographical ones. One race, for instance, may live in woodland, while another may prefer prairie or open fields. Such variants may occur in the same locality with little intergradation. An example of this is *Myrmica sabuleti trullicornis* n. subsp., a woodland form, whose closest relative is *Myrmica sabuleti americana* Weber, a prairie form.

No forms or records have been included in the list unless the specimens were seen and studied by the author. All collections were made by the author unless otherwise stated. Holotypes of the new species are in the author's collection, and paratypes will be given to the National Museum, Iowa State College, and other institutions and individuals.

For entomologists who may wish to identify Iowa ants, keys for the separation of all forms listed are included.

KEY TO SUBFAMILIES OF FORMICIDAE

1. Pedicel of abdomen 2-jointed2
 Pedicel of abdomen 1-jointed3
2. Frontal carinae usually somewhat separated and at least partially covering the antennal insertions3. *Myrmicinae*
 Frontal carinae approximate, linear, not at all covering antennal insertions2. *Dorylinae*
3. A distinct constriction between the first and second segments of the gaster; sting developed1. *Ponerinae*
 Without a constriction between first and second segments of gaster; sting vestigial or absent4

4. Apex of hypopygium with a circular, hair-fringed opening^a for the ejaculation of the poison5. *Formicinae*
 Hypopygium without such an opening4. *Dolichoderinae*

1. SUBFAMILY PONERINAE

KEY TO GENERA OF PONERINAE

1. Mandibles falcate, their teeth bifurcated1. *Stigmatomma*
 (one Iowa species, *S. pallipes subterranea* Creighton)
 Mandibles and their teeth normal2
 2. Tip of gaster strongly deflected ventrally and anteriorly, petiole nodiform2. *Sysphincta*
 (one Iowa species, *S. pergandei* Emery)
 Tip of gaster not bent anteriorly underneath; petiole with a large erect scale3. *Ponera*

1. *Stigmatomma*

1. *Stigmatomma pallipes subterranea* Creighton

1940 *S. pallipes subterranea* Creighton, Amer. Mus. Nov., No. 1079:8. ♀

Records: Ames, Burlington, Bellevue. Also Sioux City (C. N. Ainslie).

The writer has taken this form under rocks in woodlands at Burlington and Bellevue and in an open field at Ames. It can be found only in the spring or fall when the ground is moist. Never more than seven specimens were found at one time.

2. *Sysphincta*

1. *Sysphincta pergandei* Emery

1895 *S. pergandei* Emery, Zool. Jahrb. Syst., 8:264. ♀

Record: Bellevue.

Apparently this species is extremely rare in Iowa as it is in all parts of its range. The writer possesses only a single specimen found under a log in wooded pasture land. Much digging and searching failed to produce any more specimens. This ant is extremely hypogeic.

3. *Ponera*

KEY TO SPECIES OF PONERA

1. Middle tibial spurs more than one-half the length of hind tibial spurs; erect hairs numerous1. *P. coarctata pennsylvanica*
 Middle tibial spurs less than one-half the length of hind spurs; erect hairs sparse2. *P. trigona opacior*

^a Although some recent authors are still calling this opening the anus, Emery (1922a) has conclusively shown that the anus or cloacal opening of the Formicinae is between the hypopygium and pygidium, as in every other subfamily of Formicidae.

1. *Ponera coarctata pennsylvanica* Buckley1866 *P. pennsylvanica* Buckley, Proc. Ent. Soc. Philad., 6:171. ♀1895 *P. coarctata pennsylvanica* Emery, Zool. Jahrb. Syst., 8:287. ♀ ♀ ♂

Records: Ames, Clinton, Inwood, Muscatine, Oak Grove State Park, Sabula. Also Sioux City (C. N. Ainslie).

This species is the commonest Ponerine in Iowa. It is common near Ames and probably occurs over much of the state. My list of localities could probably be greatly expanded if a more intensive search were made for it. *P. pennsylvanica* is rather hypogeic in habit and nests in small colonies.

2. *Ponera trigona opacior* Forel1893 *P. trigona* var. *opacior* Forel, Trans. Ent. Soc. Lond., p. 363. ♀ ♀1895 *P. trigona* var. *opacior* Emery, Zool. Jahrb. Syst., 8:268. ♀ ♀ ♂

Records: Clinton, Glenwood, Little Sioux.

Iowa probably marks the northern limit of the range of this species. Since it was found only on opposite sides of the state, *opacior* has probably managed to creep sporadically northward only along the bluffs of the Mississippi and Missouri rivers.

2. SUBFAMILY DORYLINAE

1. *Eciton*

KEY TO SPECIES OF ECITON

1. Head and thorax entirely opaque.....1. *E. nigrescens*
 Head and pleurae of prothorax shining2. *E. opacithorax*

1. *Eciton (Neivamyrmex) nigrescens* (Cresson)1872 *Labidus nigrescens* Cresson, Trans. Amer. Ent. Soc., 4:194. ♂1894 *Eciton (Acamatus) schmitti* Emery, Bull. Soc. Ent. Ital., 26:183. ♀1908 *Eciton (Acamatus) nigrescens* Wheeler, Bull. Amer. Mus. Nat. Hist., 24:417. ♂1938 *Eciton (Acamatus) nigrescens* M. R. Smith, Proc. Ent. Soc. Wash., 40(6):157-160. ♀ ♂1940 *Eciton (Neivamyrmex) nigrescens* Borgmeier, Rev. de Ent. Brasil, 11:606.

Records: Little Sioux, Sioux City. Also Sioux City (C. N. Ainslie).

This species can be found in Iowa only along the bluffs of the Missouri River. After rains they may be found marching in long columns. Sioux City is the farthest north that any Doryline ant has ever been taken, but since they appear to closely follow the Missouri River bluffs, it is quite possible that the range of *nigrescens* extends into South Dakota.

2. *Eciton (Neivamyrmex) opacithorax* Emery1894 *E. (Acamatus) californicum opacithorax* Emery, Bull. Soc. Ent. Ital., 26:184. ♀1900 *E. (Acamatus) opacithorax* Emery, Mém. Accad. Sci. Bologna, 8(5):23 ♀1901 *E. (Acamatus) opacithorax* Wheeler and Long, Amer. Natur., 35:163, 173. ♀ ♂

Record: Glenwood.

This species, found accidentally, was tunneling an inch or two beneath the ground. This is the farthest north this species has been taken. It is rarer than *nigrescens*, but it is quite possible that its range extends as far north.

3. SUBFAMILY MYRMICINAE

KEY TO GENERA OF MYRMICINAE

1. Postpetiole articulated to dorsal surface of gaster, which is flattened dorsally, more convex ventrally, and pointed at the tip 7. *Crematogaster*
Postpetiole articulated to anterior end of gaster, which is of a different shape ... 2
2. Antennae 6-jointed; the scapes retractible into long scrobes; head cordiform 11. *Strumigenys*
Antennae with more than six joints 3
3. Antennae 10-jointed, funicular clubs 2-jointed. 9. *Solenopsis*
[one Iowa species, *S. molesta* (Say)] 4
Antennae different 4
4. Epinotum with two pairs of spines (anterior pair feeble and dorsally projecting) 10. *Myrmecina*
(one Iowa species, *M. graminicola americana* Emery) 5
Epinotum different 5
5. Last three joints of the funiculus forming a club as long as or longer than the remainder 6
Last three joints not as long as the remainder of the funiculus, although the last three joints may form an indistinct club 8
6. Thorax without any trace of teeth or spines 8. *Monomorium*
Epinotum with at least feeble spines; integument often strongly sculptured 7
7. Workers strongly dimorphic, usually without intermediates; scapes of minor workers reaching beyond the head 5. *Pheidole*
Workers monomorphic; scapes usually not reaching the hind border of the head 6. *Leptothorax*
8. Gula with a basket of long hairs 2. *Pogonomyrmex*
[one Iowa species, *P. occidentalis* (Cresson)] 9
Gula with only normal hairs 9
9. Posterior tibial spurs pectinated; head and thorax strongly rugose ... 1. *Myrmica*
Posterior tibial spurs simple 10
10. Small hypogeic species with vestigial eyes and two keels on the clypeus ... 3. *Stenamma*
Medium-sized epigeic species with well-developed eyes and no keels on the clypeus 4. *Aphaenogaster*

1. *Myrmica*

KEY TO SPECIES OF MYRMICA

1. Scape without a lobe or lamina at the bend near the base; gaster punctate 4. *M. punctiventris*
Scape with a lobe or lamina at the bend 2
2. Lamina of the scape carried partially around the bend and then ventrad along the medial side of the base of the scape; postpetiole convex beneath in profile. 3. *M. schencki emeryana*
Lamina carried completely around the bend and attached to both sides of the scape distal to the bend; postpetiole straight beneath 3
3. Lamina of the scape produced into a large spoon-shaped lobe 1. *M. sabuleti trullicornis*
Lamina of the scape not produced into a lobe 2. *M. sabuleti americana*

1. *Myrmica sabuleti* subsp. *trullicornis* n. subsp.

WORKER. Length about 5.5 mm.

Head, excluding the mandibles, slightly (about one-twentieth) longer

than broad, with moderately convex sides, and feebly convex or slightly excised posterior border. Frontal carinae produced into large lobes projecting dorso-laterally from the head, strongly converging behind. Scape bent at right angles near the base, the bend fitted on the dorsal side with a relatively enormous lobe much larger than in any other previously described North American form except *schencki spatulata* M. R. Smith. When seen from above, this lobe is rather circular in outline and distinctly concave so that it appears much like a ladle. Its very sharp edges are produced along each side of the scape for a short distance.

Thorax with obtuse mesoepinotal impression. Epinotal spines about one-half again as long (about .38 mm. long) as the distance between their bases. In profile the petiole as high as the distance between its ventral tooth and the postpetiole; dorsal surface of petiolar node nearly straight for a short distance before dropping abruptly behind. Postpetiole six-sevenths as long as high, with very convex dorsum in profile, ventral surface nearly straight as in *sabuleti americana* Weber.

Clypeus and mandibles longitudinally striate. Frontal area striatopunctate. Front with about 13 strong longitudinal striae which tend to diverge behind and fuse with the strong reticulate sculpture of the rest of the head. Thorax longitudinally rugose, the rugae larger, and more vermiculate on the pronotum than behind. On the pleurae of the epinotum the rugae converge toward and disappear upon the epinotal spines, about 8-10 rugae taking part in this effect on each side. Dorsa and pleurae of petiole and postpetiole rugose, more irregularly and not so deeply rugose on the dorsa. Gaster smooth and shining.

Pilosity much as in *americana*, the erect hairs moderately abundant, those on the occiput and thorax with blunt tips. Hairs on the scapes oblique, those on the legs subappressed to oblique. Pubescence sparse on all parts.

Color blackish brown; dorsum of the head and gaster darker than the other regions.

FEMALE. Not differing appreciably from the female of *americana* Weber except in having large ladle-shaped lobes on the scapes, somewhat finer sculpture, and darker color.

Described from 34 workers collected April 30, 1941, 4 workers collected August 5, 1939, from woodland colonies near Ames (type locality), and 11 workers and a female from woods near Boone, collected May 3, 1941.

The very similar manner in which the ladle-shaped lobes are attached to the scapes, the almost identical shape of the postpetiole, and many other similarities, show *trullicornis* to be most closely related to *americana* Weber. *M. trullicornis* may be distinguished from *americana* by the large lobe on the scape, which in *americana* is produced only as a small lamina curved around the bend. The sculpture of *trullicornis* seems finer, and the color is darker. *M. trullicornis* also seems to be a woodland form whereas *americana* prefers prairies and open fields. The writer possesses

a series of workers from Sauk Rapids, Minnesota, showing well-marked intergradation between *trullicornis* and *americana*. These forms therefore seem to be no more than subspecifically distinct.

The placement of *trullicornis* and *americana* under *sabuleti* Meinert seems somewhat incongruous as the lamina of the scape is quite differently constructed in this species. Possibly *americana* will prove specifically distinct from *sabuleti*, and *trullicornis* can then be placed as a subspecies of *americana*, and considered as an ecological race of it.

M. sabuleti trullicornis should not be confused with *M. schencki spatulata* M. R. Smith. The frontal carinae are not at all produced into lobes in *spatulata*, the shape and attachment of the large lobe on the scape are quite different, the sculpture is coarser, and the postpetiole is convex beneath.

2. *Myrmica sabuleti americana* Weber

1939 *M. sabuleti americana* Weber, Lloydia, 2:144.

Records: Ames, Boone, Clinton, Keokuk, Jewell, Oak Grove State Park, Granite. Also Sioux City (C. N. Ainslie).

This ant seems common all over Iowa. It prefers to nest in open fields.

3. *Myrmica schencki emeryana* Forel

1914 *M. scabrinodis schencki* var. *emeryana* Forel, Deutsche Ent. Zeitschr., p. 617. ♀ ♀ ♂

Records: Ames, Spirit Lake, Boone, Clinton, Sabula, Inwood.

This ant is fairly common in woodlands in Iowa.

4. *Myrmica punctiventris* Roger

1863 *M. punctiventris* Roger, Berlin Ent. Zeitschr., 7:190. ♀

1886 *M. punctiventris* Mayr, Verh. Zool.-bot. Ges. Wien, 36:450. ♀ ♀

1895 *M. punctiventris* Emery, Zool. Jahrb. Syst., 8:312. ♂

Record: Belle Plaine.

M. punctiventris prefers to live in dense woodlands and is probably much rarer in Iowa than in the eastern states.

2. *Pogonomyrmex*

1. *Pogonomyrmex (Pogonomyrmex) occidentalis* (Cresson)

1865 *Myrmica occidentalis* Cresson, Proc. Ent. Soc. Philad., 4:426. ♀ ♀

1882 *Pogonomyrmex occidentalis* McCook, The Honey Ant, etc., p. 123-162. ♀ ♀ ♂

Record: Sioux City (C. N. Ainslie).

This record may be the result of mislabeling. The writer has failed to take this species in Iowa, even along the Missouri River bluffs area. It is, of course, possible that it may sporadically occur in this area.

3. *Stenamma*

KEY TO SPECIES OF STENAMMA

1. Eyes with approximately 30 facets, head and thorax uniformly opaque 1. *S. brevicorne*
 Eyes with about 15 facets; pronotum somewhat shining; mesoepinotal impression deeper 2. *S. brevicorne impressum*

1. *Stenamma brevicorne* (Mayr)

1886 *Aphaenogaster brevicorne* Mayr, Verh. Zool.-bot. Ges. Wien, 36:447. ♀ ♀

1895 *Stenamma brevicorne* Emery, Zool. Jahrb. Syst., 8:298. ♀ ♀ ♂

Records: Ames, Clinton, McGregor, DeWitt. Also Arnolds Park (Judson McQuire); Sioux City (C. N. Ainslie).

This ant is probably common in woodlands over much of the state. It is hypogeic in habit. The winged forms apparently overwinter in the nests as adults or pupae, as they may be found in the nests in early spring.

2. *Stenamma brevicorne impressum* Emery

1895 *S. westwoodi diecki* var. *impressum* Emery, Zool. Jahrb. Syst., 8:301. ♀ ♀

1901 *S. brevicorne diecki* var. *impressum* Forel, Ann. Soc. Ent. Belg., 45:347.

Record: Tama.

As this subspecies is represented by only one specimen, it must be a rare form in Iowa. The specimens which the author previously referred to *impressum* (1941a) belong to the typical *brevicorne*.

4. *Aphaenogaster*

KEY TO SPECIES OF APHAENOASTER

1. Scape with a flattened lobe at the base 5. *A. treatae*
 Scape without a lobe at the base 2
2. Basal third of first gastric segment striate 3. *A. mariae*
 First gastric segment not striate 3
3. Epinotal spines longer than the base of the epinotum; few or no hairs on gaster; color deep red 4. *A. tennesseensis*
 Epinotal hairs much shorter than the base of the epinotum; gaster hairy; reddish brown to black 4
4. Reddish brown 1. *A. fulva aquia*
 Deep brown or black 2. *A. fulva picea*

1. *Aphaenogaster (Attomyrma) fulva aquia* (Buckley)

1867 *Myrmica (Monomorium) aquia* Buckley, Proc. Ent. Soc. Philad., 6:431. ♂

1895 *Stenamma (Aphaenogaster) fulva aquia* Emery, Zool. Jahrb. Syst., 8:304. ♀ ♀ ♂

1922 *Aphaenogaster (Attomyrma) fulva aquia* Emery, Gen. Insec., fasc. 174:57.

Records: Ames, Boone, Holy Cross, Clinton, Dubuque.

Common in all wooded portions of the state.

2. *Aphaenogaster (Attomyrma) fulva picea* Emery

1895 *Stenamma (Aphaenogaster) fulva aquia* var. *picea* Emery, Zool. Jahrb. Syst., 8:305. ♀ ♀ ♂

- 1922 *Aphaenogaster (Attomyrma) fulva aquia* var. *picea* Emery, Gen. Insec., fasc. 174:57.

Records: Ames, Backbone State Park, Glenwood, Waubonsie State Park, Oak Grove State Park, Clinton.

This variant does not seem very distinct. Perhaps it may be more distinct in the Eastern States but intergrades with *aquia* rather readily in Iowa. Its colonies always seem rather depauperate compared with those of *aquia*.

3. *Aphaenogaster (Attomyrma) mariae* Forel

- 1886 *Aph. mariae* Forel, Ann. Soc. Ent. Belg., 30(C.R.):41. ♀

Record: Ames.

The host of this ant is *Aphaenogaster fulva aquia*. The writer on three occasions found *mariae* with *aquia* near Ames. It is much rarer than *tennesseensis*, a very similar and probably closely related species.

4. *Aphaenogaster (Attomyrma) tennesseensis* (Mayr)

- 1862 *Atta tenneseensis* Mayr, Verh. Zool.-bot. Ges. Wien, 12:743. ♂
1922 *Aphaenogaster (Attomyrma) tenneseensis* Emery, Gen. Insec., fasc. 174:60.

Records: Ames, Oak Grove State Park, Rice Lake State Park, Clinton, Belle Plaine, Denison, Boone. Also Sioux City (C. N. Ainslie).

The temporary host of this ant is *A. fulva aquia*, with which it is occasionally found under rocks. When the colonies are fully developed they can be found only in rotting wood.

5. *Aphaenogaster (Attomyrma) treatae* Forel

- 1886 *Aph. treatae* Forel, Ann. Soc. Ent. Belg., 30(C.R.):40 ♂ ♀ ♂

Records: Little Sioux, Glenwood, Sioux City, Princeton, DeWitt.

As has been stated in the introduction, this species seems to have a discontinuous distribution in Iowa, occurring only in the extreme eastern and western parts of the state.

5. Pheidole

KEY TO SPECIES OF PHEIDOLE

1. Thorax and gaster with only sparse, clavate hairs 3. *P. sitarches*
Thorax and gaster with numerous, slender hairs 2
2. Soldiers with occipital lobes of head shining; workers with shining heads
..... 1. *P. bicarinata*
Occipital lobes of soldiers reticulate-rugose; workers with heads punctate and
opaque 2. *P. pilifera*

1. *Pheidole (Pheidole) bicarinata* Mayr

- 1870 *Ph. bicarinata* Mayr, Verh. Zool.-bot. Ges. Wien, 20:982, 989. 24

Records: Clinton, Ames, Akron, Burlington, Oak Grove State Park, McGregor, Hinton. Also Sioux City (C. N. Ainslie).

This species is common over most of Iowa. It seems to thrive well in our cities and towns, even though it was originally a member of the prairie fauna. It is less granivorous than *pilifera*.

In the author's preliminary list (1941a) *bicarinata* was misidentified as *P. vinelandica* Forel, a closely related species.

2. *Pheidole (Pheidole) pilifera* (Roger)

1863 *Leptothorax pilifer* Roger, Berlin Ent. Zeitschr., 7:180. ♀

1886 *Pheidole pennsylvanica* Mayr, Verh. Zool.-bot. Ges. Wien, 36:455. 21 ♀ ♀ ♂

1895 *Pheidole pilifera* Emery, Zool. Jahrb. Syst., 8:290.

Records: Ames, Bellevue, DeWitt, Princeton.

This ant is numerous in prairie lands. It does not thrive well in our cities and towns, but the writer has occasionally seen it in such situations. *P. pilifera* will accept dead insects if offered, but is largely granivorous.

3. *Pheidole (Pheidole) sitarches* Wheeler

1908 *Ph. sitarches* Wheeler, Bull. Amer. Mus. Nat. Hist., 24:440. 21 ♀ ♀

Record: Glenwood.

This species was originally described from Texas and apparently occurs in Iowa only in the extreme southwestern part of the state along the bluffs of the Missouri River.

6. *Leptothorax*

KEY TO SPECIES OF LEPTOTHORAX

1. Antennae 11-jointed2
- Antennae 12-jointed6
2. Thorax with a faint but distinct mesoepinotal constriction (subgenus *Mychothorax*)6. *L. acervorum canadensis* Provancher
- Thorax without a mesoepinotal constriction3
3. Epinotal spines very short, dentiform; color black....4. *L. fortinodis melanotica*
- Epinotal spines longer4
4. Head shining; color black.....3. *L. longispinosus laeviceps*
- Head sculptured; color yellow5
5. Spines long and curved, their bases approximate.....1. *L. curvispinosus*
- Spines shorter and straight, their bases farther apart.....2. *L. ambiguus*
6. Without mesoepinotal constriction; postpetiole much broader than petiole....5. *L. tricarinatus*
- With deep mesoepinotal constriction; petiole pedunculate (subgenus *Dichothorax*)7. *L. pergandei*

1. *Leptothorax (Leptothorax) curvispinosus* Mayr

1886 *L. curvispinosus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:451, 453. ♂ ♀

1886 *L. curvispinosus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:451, 453. ♂ ♀

Records: Ames, Clinton, Tama, Waubonsie State Park, Belle Plaine, Denison, Granite. Also Sioux City (C. N. Ainslie).

A common woodland form. Several colonies have been found nesting in dried hollow stems of plants near Ames.

2. *Leptothorax (Leptothorax) ambiguus* Emery

- 1895 *L. curvispinosus ambiguus* Emery, Zool. Jahrb. Syst., 8:320. ♂
1940 *L. ambiguus* Wesson and Wesson, Amer. Midl. Nat., 24(1):97.

Records: Boone, Ames.

A much rarer ant than *curvispinosus*. No nests were found. All specimens were either caught in sweeping or with an aspirator as they were crawling on the ground.

3. *Leptothorax (Leptothorax) longispinosus* subsp. *laeviceps* n. subsp.

WORKER. Length, 2.2 mm.

Head oblongate, one-eighth longer than broad, excluding the mandibles. Antennae 11-jointed; scapes nearly reaching the posterior corners of the head. Funicular joints 2-7 broader than long; club one-fifth longer than rest of funiculus. Dorsum of thorax moderately and evenly convex in profile. Epinotal spines long, straight, sharp, somewhat diverging seen from above, projecting backwards and slightly upward, about one-third as long as the distance from bases to neck of pronotum. Petiole and postpetiole much as in *longispinosus* s. str. Petiole a little larger in profile than postpetiole, the antero-ventral spine very weak, node bluntly subtriangular in profile and not quite as high as in typical *longispinosus*.

Head smooth and shining except for the cheeks, which are striate. Median lobe of clypeus rather indistinctly striate. Thorax striato-punctate, especially on the pleurae; the dorsum feebly shining. The interrugal spaces of the thorax are distinctly wider than in typical *longispinosus*. Petiole and postpetiole punctate.

Erect hairs somewhat clavate, arranged as in typical *longispinosus*. Dark brownish black.

Described from 12 specimens found under a stone on the high Mississippi River bluffs near McGregor, Iowa, June 10, 1940. The typical *longispinosus* apparently does not range as far west as Iowa. *L. laeviceps* may therefore be regarded as a depauperate, geographical race.

L. laeviceps differs from *longispinosus* s. str. in being smaller, and having the sculpturing distinctly less coarse on all parts. The striae on the thorax are farther apart, leaving room for large interrugal punctures. The dorsum of the thorax is a little more convex in profile, and the epinotal spines project a little upward rather than being horizontal. The petiole and postpetiole are not quite as robust. The color of *longispinosus* is often pitch black. The sculpture of the head of *laeviceps* is probably much like that of *L. schmittii* Wheeler, but this species has 12-jointed antennae and much shorter epinotal spines.

4. *Leptothorax (Leptothorax) fortinodis melanotica* Wheeler

- 1903 *L. fortinodis* var. *melanotica* Wheeler, Proc. Acad. Nat. Sci. Philad., 55:235. ♂ ♀
1940 *L. schaumii* var. *fortinodis* Wesson and Wesson, Amer. Midl. Nat., 24(1):96.

Records: Ames, DeWitt, Clinton.

As Wesson and Wesson have stated (*loc. cit.*) this form may be synonymous with the typical *fortinodis* Mayr, which in turn may be no more than a subspecies of *schaumi* Roger. The author would point out, however, that even though specimens referable to *melanotica* may intergrade indistinguishably with *fortinodis* in the East, *melanotica* may be a valid geographical race in the Middle West, from which region it was described. All the author's specimens are pitch black as described.

5. *Leptothorax (Leptothorax) tricarinatus* Emery

1895 *L. tricarinatus* Emery, Zool. Jahrb. Syst., 8:321. ♀

Records: Inwood, Oak Grove State Park. Also Sioux City (C. N. Ainslie).

This species may be distinguished immediately from all the other species of typical *Leptothorax* in Iowa by its 12-jointed antennae and large postpetiole. *L. tricarinatus* is not related to the members of the subgenus *Dichothorax* which also have 12-jointed antennae.

L. tricarinatus nests in the ground in small colonies.

6. *Leptothorax (Mychothorax) acervorum canadensis* Provancher

1887 *L. canadensis* Provancher, Addit. Faune Canada, Hym., p. 245. ♂ ♀ ♂

1903 *L. acervorum canadensis* Wheeler, Proc. Acad. Nat. Sci. Philad., 55:225. ♂ ♀

Record: Spirit Lake.

This species was found nesting under the bark of a log. *L. acervorum canadensis* is a boreal species, apparently rare in Iowa even in the northern part.

7. *Leptothorax (Dichothorax) pergandei* Emery

1895 *L. (D.) pergandei* Emery, Zool. Jahrb. Syst., 8:318, 323. ♀ ♀ ♂

Records: Boone, Elkader, Glenwood, Bellevue, Dubuque, Sabula.

This ant nests in soil on sunny hillsides. It seems more xerophilous and moves more rapidly than the species of typical *Leptothorax*. It is common nowhere but, nevertheless, cannot be considered very rare in Iowa.

7. Crematogaster

KEY TO SPECIES OF CREMATOGASTER

1. Black in color; thorax opaque (subgenus *Crematogaster* s. str.) 1. *C. lineolata* (Say)
- Mostly yellow; thorax shining (subgenus *Orthocrema*) 2. *C. minutissima missouriensis* Emery

1. *Crematogaster (Crematogaster) lineolata* (Say)

1836 *Myrmica lineolata* Say, Boston Jour. Nat. Hist., 1:290. ♀ ♀ ♂

1863 *Crematogaster lineolata* Roger, Verz. Formicid., p. 37.

Records: Ames, Mt. Vernon, Sabula, Keokuk, Muscatine, McGregor, Dubuque, Glenwood.

This species occurs all over the state but does not seem especially common.

2. *Crematogaster (Orthocrema) minutissima missouriensis* Emery

1895 *C. victima missouriensis* Emery, Zool. Jahrb. Syst., 8:288 (in footnote). ♂

1939 *C. (O.) minutissima missouriensis* Creighton, Psyche, 46(4):138.

Records: Little Sioux, Glenwood, Sioux City. Also Sioux City (C. N. Ainslie).

This ant is abundant along the Missouri River bluffs but lacking in other parts of the state. It nests in the ground in small colonies.

8. Monomorium

KEY TO SPECIES OF MONOMORIUM

1. Black; all surfaces shining.....1. *M. minimum*
 Yellow; head and thorax finely reticulate-punctate 2. *M. pharaonis*

1. *Monomorium (Monomorium) minimum* (Buckley)

1867 *Myrmica (Monomorium) minima* Buckley, Proc. Ent. Soc. Philad., 6:338. ♀ ♀

1895 *Monomorium minutum* var. *minimum* Emery, Zool. Jahrb. Syst., 8:274. ♀ ♀ ♂

1914 *Monomorium minimum* Wheeler, Jour. New York Ent. Soc., 22:42.

Records: Little Sioux, Inwood, Tama, Ames, Boone. Also Sioux City (C. N. Ainslie).

This minute species usually builds small crater nests in the ground. The writer has once taken it from beneath the bark of a log.

2. *Monomorium (Monomorium) pharaonis* (Linné)

1758 *Formica pharaonis* Linné, Syst. Nat., ed. 10, 1:580.

1862 *Monomorium pharaonis* Mayr, Verh. Zool.-bot. Ges. Wien, 12:752.

Record: Ames.

This species does not live out-of-doors in these latitudes. It is occasionally found in buildings and houses, where it apparently nests in the walls.

9. Solenopsis

1. *Solenopsis (Diplorhoptrum) molesta* (Say)

1836 *Myrmica molesta* Say, Boston, Jour. Nat. Hist., 1:293. ♀

1895 *Solenopsis molesta* Emery, Zool. Jahrb. Syst., 8:277. ♀ ♀ ♂

Records: Ames, Sioux City, Boone, Marshalltown, Inwood, Tama, Belle Plaine. Also Sioux City (C. N. Ainslie).

Probably very abundant over the entire state.

10. Myrmecina

1. *Myrmecina graminicola americana* Emery1895 *M. latreillei americana* Emery, Zool. Jahrb. Syst., 8:271. ♀1922 *M. graminicola americana* Emery, Gen. Insec., fasc. 174:232.

Records: DeWitt, Clinton, Ames, Boone.

Winged males were found in a nest in late August. This ant is strictly hypogeic.

M. americana differs from the typical European *graminicola* rather distinctly. The scapes of *americana* are not flattened and broad at the base but are circular in cross section; the clypeal teeth are less distinct and the median clypeal carina nearly absent, the head is slightly broader than long rather than a little longer than broad, and is also distinctly excised behind. The thorax is a little broader in proportion to its length, and the anterior epinotal spines are better developed. *M. americana* may thus deserve to rank as a good species.

11. Strumigenys

KEY TO SPECIES OF STRUMIGENYS

1. Visible portion of mandibles one-third as long as the head, a basal tooth visible just before the clypeus1. *S. pergandei*
- Visible portion of mandibles one-fifth as long as the head, no basal tooth visible2. *S. pulchella*

1. *Strumigenys (Cephaloxys) pergandei* Emery1885 *S. pergandei* Emery, Zool. Jahrb. Syst., 8:326. ♀ ♀ ♂1931 *S. (C.) pergandei* M. R. Smith, Ann. Ent. Soc. Amer., 24(4):698.

Records: Boone, Holy Cross, Bellevue.

A rare species in Iowa. It is usually found near the nests of other ants.

2. *Strumigenys (Cephaloxys) pulchella* Emery1895 *S. pulchella* Emery, Zool. Jahrb. Syst., 8:327. ♀1931 *S. (C.) pulchella* M. R. Smith, Ann. Ent. Soc. Amer., 24(4):702. ♀

Record: Ames.

This species is either extremely rare or extremely hypogeic in Iowa. In the spring, single workers can rarely be found under rocks in damp soil.

4. SUBFAMILY DOLICHODERINAE

KEY TO GENERA OF DOLICHODERINAE

1. Epinotum with a conical point2. *Dorymyrmex*
- Epinotum without a conical point2

2. Petiolar scale very small and strongly inclined forward, not distinct. .3. *Tapinoma*
 [one Iowa species, *T. sessile* (Say)]
 Petiolar scale distinct, more erect, sharply pointed above.1. *Iridomyrmex*
 [one Iowa species, *I. pruinose analis* (Ern. André)]

1. *Iridomyrmex*

1. *Iridomyrmex pruinose analis* (Ern. André)

- 1893 *Tapinoma anale* Ern. André, Rev. Entom., p. 148. ♀
 1895 *Tapinoma pruinose* var. *anale* Emery, Zool. Jahrb. Syst., 8:333.
 1912 *Iridomyrmex analis* Emery, Gen. Insec., fasc. 137:26.

Records: Glenwood, Inwood, Oak Grove State Park, Little Sioux. Also Sioux City (C. N. Ainslie).

This species is common along the Missouri River bluffs and also in prairie remnants in the area drained by the Missouri River system. It does not occur in central or eastern Iowa.

2. *Dorymyrmex*

KEY TO SPECIES OF DORYMYRMEX

1. Head and thorax brown, lighter than gaster.1. *D. pyramicus*
 Color uniformly black2. *D. pyramicus niger*

1. *Dorymyrmex pyramicus* (Roger)

- 1863 *Prenolepis pyramica* Roger, Berl. Ent. Zeitschr., 7:160. ♀
 1886 *Dorymyrmex pyramicus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:365, 433. ♀♀
 1895 *Dorymyrmex pyramicus* Emery, Zool. Jahrb. Syst., 8:331. ♂

Records: Sioux City, Little Sioux, Oak Grove State Park, Inwood. Also Sioux City (C. N. Ainslie).

The distribution of this species in Iowa is the same as that of *Iridomyrmex analis*. Some of the author's specimens seem somewhat transitional to *D. pyramicus niger*.

2. *Dorymyrmex pyramicus niger* Pergande

- 1895 *D. pyramicus* var. *niger* Pergande, Proc. Calif. Acad. Sci., 5(2):871.

Record: Ames:

Besides the darker, uniform color of the specimens the writer has referred to *niger*, there are also these differences between them and typical *pyramicus*. The head of *niger* is a little more elongate, the scapes slightly longer, the second funicular joint shorter, the mesoepinotal suture not deeply impressed, and the petiole smaller and blunter.

This form nests only in virgin prairie or open fields. On a virgin prairie remnant near Ames it was especially abundant, more abundant than any other ant. It seems probable that this form was a dominant species in the original prairie fauna of Iowa before cultivation extinguished it. *D. niger* can be found occasionally in pasture lands but does

ants in excreta of shrews from the vicinity of Ames. It is probably a common species but because of its extremely small size and hypogeic habits, not often found.

2. *Camponotus*

KEY TO SPECIES OF CAMPONOTUS

1. Clypeus entire anteriorly or only very broadly notched, large species up to 13 mm.....2
Clypeus notched; small species up to 7 mm. (subgenus *Myrmentoma*).....5
2. Head of major worker longer than wide; body somewhat shining.....3
Head of major worker wider than long; body more opaque.....4
3. Head black, thorax and gaster dark brown4. *C. castaneus americanus*
Head dark brown; thorax and gaster tan, scarcely infuscated.....3. *C. castaneus*
4. Thorax black, gaster with long pubescence1. *C. herculeanus pennsylvanicus*
Thorax red, gaster with only short pubescence..2. *C. herculeanus novaeboracensis*
5. Cheeks and clypeus with elongate, piligerous foveolae6
Cheeks and clypeus without elongate, piligerous foveolae7
6. Head and thorax largely black or dark brown.....8. *C. caryae subbarbatus*
Head and thorax red7. *C. caryae discolor*
7. Head and thorax largely black5. *C. caryae nearcticus*
Head and thorax red6. *C. caryae rasilis*

1. *Camponotus (Camponotus) herculeanus pennsylvanicus* (Degeer)

1773 *Formica pennsylvanica* Degeer, Mém. Hist. Insect., 3:603. ♂♀♂

1879 *Camponotus herculeanus pennsylvanicus* Forel, Bull. Soc. Vaud. Sci. Nat., 16:57.

Records: Ames, Princeton, Little Sioux, Glenwood, Sioux City, Ruthven. Also Sioux City (*C. N. Ainslie*).

This species always lives in galleries which it excavates in solid or rotten wood. It occasionally nests in the beams of frame houses, weakening them considerably. Incipient colonies consisting of a female and several minor workers can often be found just under the bark of logs. The above list of localities could be considerably lengthened as *pennsylvanicus* is common in every woodland.

2. *Camponotus (Camponotus) herculeanus novaeboracensis* (Fitch)

1854 *Formica novaeboracensis* Fitch, Trans. New York State Agric. Soc., 14:52. ♂

1910 *Camponotus herculeanus ligniperda* var. *noveboracensis* Wheeler, Ann. New York Acad. Sc., 20:340. ♂ ♀♂

1925 *Camponotus (C.) herculeanus* var. *novaeboracensis* Emery, Gen. Insec., fasc. 183:72.

Records: Ames, Estherville, Holy Cross, Spirit Lake, Backbone State Park, Rice Lake State Park. Also Indianola (*D. T. Jones*).

This ant nests in wood as *pennsylvanicus* does. It appears to have a more boreal distribution than *pennsylvanicus* and does not occur in the southern part of Iowa. *C. novaeboracensis* and *pennsylvanicus* sometimes occur in the same locality, apparently without intergradation. It seems, therefore, that they could be considered specifically, rather than only subspecifically distinct.

3. *Camponotus* (*Camponotus*) *castaneus* (Latreille)

1802 *Formica castanea* Latreille, fourmis, p. 118. ♀ ♀ ♂

1886 *Camponotus castaneus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:420.

Record: Burlington.

This southern species seems to reach its northern limit in south-eastern Iowa. The colony the writer found was nesting under a flat rock in woodland.

4. *Camponotus* (*Camponotus*) *castaneus americanus* Mayr

1862. *C. americanus* Mayr, Verh. Zool.-bot. Ges. Wien, 12:661. ♀ ♀

1893 *C. castaneus americanus* Emery, Zool. Jahrb. Syst., 7:674.

Records: Ames, Clinton, Backbone State Park, Inwood.

This ant nests in the ground, never in wood. It prefers woodlands, however. Winged males and females were taken in nests in early April and May, so evidently these casts overwinter as adults. The winged casts of *herculeanus pennsylvanicus* often overwinter as adults, also.

5. *Camponotus* (*Myrmentoma*) *caryae nearcticus* Emery

1893 *C. marginatus* var. *nearcticus* Emery, Zool. Jahrb. Syst., 7:675. ♀ ♀

1910 *C. fallax* var. *nearcticus* Wheeler, Jour. New York Acad. Sci., 18:222. ♀ ♀ ♂

1917 *C. (Camponotus) caryae* Wheeler, Psyche, 24:27.

Records: Ames, Tama, Holy Cross, Clinton.

This ant nests in the dead branches of hickory and oak trees. Specimens are not often taken, but it is probably a fairly common woodland form in Iowa.

6. *Camponotus* (*Myrmentoma*) *caryae rasilis* Wheeler

1910 *C. fallax rasilis* Wheeler, Jour. New York Ent. Soc., 18:227. ♀ ♀ ♂

1917 *C. caryae rasilis* Wheeler, Psyche, 24:28.

Record: Sioux City (C. N. Ainslie).

This is another of the southern forms which appear to have extended their range northward along the Missouri River bluffs. The Iowa specimens of *rasilis* collected by Ainslie are all much smaller than the typical *rasilis* of the southern states.

7. *Camponotus* (*Myrmentoma*) *caryae discolor* (Buckley)

1866 *Formica discolor* Buckley, Proc. Ent. Soc. Philad., 6:166. ♀ ♀

1893 *Camponotus marginatus discolor* Emery, Zool. Jahrb. Syst., 7:277. ♀ ♀ ♂

1917 *Camponotus caryae discolor* Wheeler, Psyche, 24:28.

Records: Ames, Boone.

This subspecies apparently has the same nesting habits as *nearcticus*. It is rarer in Iowa than *nearcticus*.

8. *Camponotus* (*Myrmentoma*) *caryae subbarbatus* Emery

1893 *C. marginatus subbarbatus* Emery, Zool. Jahrb. Syst., 7:676: ♀ ♀ ♂

1917 *C. caryae subbarbatus* Wheeler, Psyche, 24:28.

Records: Ames, Boone.

The two colonies of this rare ant that the writer has found have been under or in rotting wood in the ground. It may thus prove to have different nesting habits than the tree-dwelling *nearcticus* and *discolor*.

Several forms of *caryae* other than the four above were included in the writer's preliminary list. These were based on old, faded or otherwise poor material reposing in the Iowa State College collection. The author has decided that their identifications are too doubtful to be included in the present paper.

3. Paratrechina

KEY TO SPECIES OF PARATRECHINA

1. Scapes with erect hairs; yellow1. *P. arenivaga*
Scapes without erect hairs; black2. *P. parvula*

1. *Paratrechina (Nylanderia) arenivaga* (Wheeler)

- 1905 *Prenolepis arenivaga* Wheeler, Bull. Amer. Mus. Nat. Hist., 21:391. ♀ ♂
1925 *Paratrechina (N.) arenivaga* Emery, Gen. Insec., fasc. 183:221.

Records: Sioux City, Blencoe, Little Sioux.

A rather common member of the Missouri River bluff fauna but not found in any other part of the state. It builds small crater nests in the loess soil of these bluffs.

2. *Paratrechina (Nylanderia) parvula* (Mayr)

- 1870 *Prenolepis parvula* Mayr, Verh. Zool.-bot. Ges. Wien, 20:948. ♀ ♂
1925 *Paratrechina (N.) parvula* Emery, Gen. Insec., fasc. 183:222.

Records: Ames, Clinton, Inwood, Dubuque, DeWitt.

This species is fairly common in Iowa. It usually nests under rocks in sunny places. The sexual phases apparently overwinter in the nests since they may be found in the nests in early spring.

4. Prenolepis

1. *Prenolepis imparis* (Say)

- 1836 *Formica imparis* Say, Boston Jour. Nat. Hist., 1:287. ♀ ♂
1886 *Prenolepis imparis* Mayr, Verh. Zool.-bot. Ges. Wien, 36:431.

Records: Ames, Backbone State Park, Clinton.

The sexual casts of *P. imparis* overwinter in the nest and fly in the first warm days of spring. This ant is common in woodlands and also in our cities and towns. It rarely appears above ground except in cool, damp weather.

5. *Lasius*KEY TO SPECIES OF *LASIUS*

1. Maxillary palpi 6-jointed2
 Maxillary palpi 3-jointed (subgenus *Acanthomyops*)7
2. Last three joints of maxillary palpi subequal in length; eyes large3
 Last two joints shorter than the fourth joint; eyes small4
3. Erect hairs present on the scapes1. *L. niger neoniger*
 Erect hairs lacking on the scapes2. *L. niger americanus*
4. Scapes not reaching the posterior corners of the head3. *L. brevicornis*
 Scapes surpassing the posterior corners of the head5
5. Scapes slightly surpassing the posterior corners of the head; last joint of
 maxillary palpi as long as the preceding joint; light yellow in color
 4. *L. flavus nearcticus*
 Scapes distinctly surpassing posterior corners; last joint of maxillary palpi
 shorter than preceding joint; color darker (subgenus *Chthonolasius*)6
6. No or very few erect hairs on gula or legs; gastric pubescence sparse revealing
 the shining surface6. *L. umbratus epinotalis*
 Erect hairs present on gula and legs; gastric pubescence dense
 5. *L. umbratus aphidicola*
7. Hairs plumose at the distal ends10. *L. plumosipilosus*
 Hairs simple or only feebly barbellate8
8. Petiole blunt; erect hairs numerous on all femora9. *L. latipes*
 Petiole sharper and notched above; erect hairs not present on all femora9
9. Scapes surpassing posterior corners of the head; penultimate joints of funiculi
 longer than broad8. *L. interjectus*
 Scapes not or scarcely surpassing posterior corners of the head; penultimate
 joints slightly broader than long7. *L. claviger*

1. *Lasius (Lasius) niger neoniger* Emery

1893 *L. niger* var. *neoniger* Emery, Zool. Jahrb. Syst., 7:639. ♀ ♀ ♂

Records: Ames, Marshalltown, Princeton, Spirit Lake.

This species could probably be found in every square mile in Iowa except along the Missouri River bluffs. It may be our commonest species.

2. *Lasius (Lasius) niger americanus* Emery

1893 *L. niger* var. *americanus* Emery, Zool. Jahrb. Syst., 7:639. ♂ ♀ ♂

1917 *L. niger alienus* var. *americanus* Wheeler, Proc. Amer. Acad. Art. Sci. Boston, 52:525.

Records: Ames, Clinton.

The paucity of records is due to neglect in collecting the species. In all probability it is at least the second commonest ant in Iowa. It does not thrive well in our cities and towns as *neoniger* does.

Lasius (Lasius) brevicornis Emery

1893 *L. brevicornis* Emery, Zool. Jahrb. Syst., 7:639. ♀ ♀ ♂

Records: McGregor, Sabula.

This species seems rare in Iowa although undoubtedly many more collections could be made in the northeastern part of the state. It does not occur near Ames.

4. *Lasius (Lasius) flavus nearcticus* Wheeler

1906 *L. flavus nearcticus* Wheeler, Psyche, 13:38.

Records: Ames, Belle Plaine, Spirit Lake.

Apparently rather rare in Iowa. It is found in woodlands under rocks or logs in moist soil. The color of this species is usually given as very light yellow with the gaster whitish. In the writer's opinion the whiteness of the gaster is caused by fading in alcohol. Although somewhat lighter than *umbratus aphidicola*, the true color of *nearcticus* is as dark as that of *brevicornis*.

5. *Lasius (Chthonolasius) umbratus aphidicola* (Walsh)

1862 *Formica aphidicola* Walsh, Proc. Ent. Soc: Philad., 1:310. ♀ ♂

1893 *Lasius umbratus mixtus* var. *aphidicola* Emery, Zool. Jahrb. Syst., 7:640. ♀ ♀ ♂

Records: Ames, Tama, Rice Lake State Park, Sabula, Belle Plaine, Clinton, Marshalltown.

The writer has found a female of *aphidicola* with a depauperate colony of *flavus nearcticus*. This seems to indicate that *nearcticus* is the host or at least an alternate host of *aphidicola*.

6. *Lasius (Chthonolasius) umbratus* subsp. *epinotalis* n. subsp.

WORKER. Length, 3.5 mm.

Head a little longer than broad, with straight posterior border and moderately convex sides. Mandibles 8-9-toothed. Scapes extending beyond posterior corners of head by about one-fourth of their length. Penultimate joints of funiculi a little longer than broad. Eyes with approximately 65 facets. Epinotum usually rounded and without a distinct angle between the base and declivity, the declivity not greatly longer than the base. Petiole cuneate in profile, ordinarily sharp and excised above. Legs rather elongate.

All surfaces shining, especially the gaster. Erect hairs somewhat thicker and longer than on *aphidicola*. Hairs on head and thorax rather long and flexuous, those on the gaster shorter, straight, and numerous. No or very few hairs on gula and legs. Pubescence moderately dense on head and thorax but not concealing the shining surface; rather sparse on the gaster.

Head and thorax sordid yellow; gaster sometimes infuscated.

Described from twenty-six specimens taken under a rock in wooded pasture land near Bellevue, Iowa, June 17, 1941.

The rounded epinotum of this subspecies is very suggestive of the species of the subgenus *Acanthomyops*. All other species of the subgenera *Lasius* s. str. and *Chthonolasius* known to the writer have a more angular epinotum with the declivity usually much longer than the base.

L. epinotalis appears closely related to *aphidicola*, but the longer, more slender antennae, less angular epinotum, smaller eyes, sparser pub-

escence, longer, less fine, rather flexuous erect hairs, which are lacking on gula and legs, and smaller size show it to be distinct.

L. umbratus speculiventris may be easily distinguished from *epinotalis* by the numerous erect hairs on scapes and legs, and lack of pubescence on the gaster. *L. umbratus subumbratus* is perhaps most closely related to *epinotalis* but differs in its larger size, more angular epinotum, somewhat more numerous hairs and pubescence, and in having hairs on the gula. The antennae of *subumbratus* are a little less slender, the eyes a little smaller, and the promesonotum a little more convex, also.

In the author's unpublished thesis this subspecies is described under the manuscript name *L. lucidiventris*.

7. *Lasius (Acanthomyops) claviger* (Roger)

1862 *Formica clavigera* Roger, Berl. Ent. Zeitschr., 6:241. ♀

1870 *Lasius (A.) claviger* Mayr, Verh. Zool.-bot. Ges. Wien, 20:950. ♀ ♀ ♂

Records: Ames, Burlington, Muscatine, Bellevue, Sabula, Boone, Belle Plaine, Inwood, Backbone State Park, Marshalltown.

This species is common in woodlands all over the Mississippi River drainage area. Very probably it is parasitic on *Lasius niger neoniger*. Wedding flights take place in late August, September, and even October. Females can sometimes be found in early spring under logs and rocks. These are always without eggs or larvae and probably are females which failed to find a suitable host colony after their wedding flight the previous fall.

8. *Lasius (Acanthomyops) interjectus* Mayr

1866 *L. (A.) interjectus* Mayr, Verh. Zool.-bot. Ges. Wien, 16:888. ♀

1886 *L. (A.) interjectus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:430. ♀ ♀ ♂

Records: Ames, Boone, Clinton. Also Sioux City (C. N. Ainslie); Des Moines (collector ?).

This species has been previously reported (Buren, 1941a) as undertaking wedding flights in warm basements in midwinter. Females taken in similar circumstances in Des Moines have been sent to the Department of Zoology and Entomology, Iowa State College. The normal wedding flight takes place in July or early August.

9. *Lasius (Acanthomyops) latipes* (Walsh)

1862 *Formica latipes* Walsh, Proc. Ent. Soc. Philad., 1:311. ♀ ♀ ♂

1866 *Lasius (A.) latipes* Mayr, Verh. Zool.-bot. Ges. Wien, 16:889.

1903 *Lasius (A.) latipes* Wheeler and McClendon, Biol. Bull., 4:149-155. Female α female β.

Records: Ames, Clinton, Spirit Lake.

This species seems rather rare in Iowa. At least the writer has had poor luck in finding it. The wedding flights take place in August, sometimes on the same day as its probable host, *Lasius niger neoniger*.

10. *Lasius (Acanthomyops) plumopilosus* Buren

1941 *L. (A.) plumopilosus* Buren, Iowa State Coll. Jour. Sci., 15 (3):231-235. ♀ ♀ ♂

Type locality: Backbone State Park.

This species may prove to be a temporary social parasite of *L. (A.) claviger*, which would make it one of the rare social hyperparasites. Since the publication of the original description the writer has failed to find this species in any other place except on the hillside where it was first found. There appear to be two or three nests of *plumopilosus* and six or more nests of *claviger* on this hillside.

6. *Formica*

KEY TO SPECIES OF FORMICA

1. Second and third funicular joints together little longer than the first, the third never longer than the penultimate; small shining species (subgenus *Proformica*)2
 Third funicular joint longer or as long as the penultimate, second and third joints together usually distinctly longer than the first; mostly medium to large-sized species; often opaque or the colors red and black.....5
2. Scapes with erect hairs.....26. *F. neogagates vetula*
 Scapes without erect hairs3
3. Gaster yellow or tan like the head and thorax.....28. *F. neogagates morbida*
 Gaster black or very dark brown4
4. Whole body black or very dark brown.....25. *F. neogagates*
 Thorax lighter than the head and gaster.....27. *F. neogagates vinculans*
5. Median joints of funiculi $1\frac{1}{2}$ times or more as long as broad; head and thorax long and slender (subgenus *Neoformica*).....6
 Median joints of funiculi less than $1\frac{1}{2}$ times as long as broad; head and thorax usually more robust (subgenus *Formica* s. str.)9
6. Erect hairs present on gula and petiole; hairs on gaster slender.....7
 Erect hairs absent on gula and petiole; hairs on gaster shorter and blunter.....8
7. Hairs on gula and petiole conspicuous; pubescence on gaster longer and denser30. *F. pallidefulva dolosa*
 Hairs often lacking on gula or petiole; pubescence on gaster shorter and sparser; color usually darker29. *F. pallidefulva incerta*
8. Head and thorax brown or reddish.....31. *F. pallidefulva nitidiventris*
 Head and thorax black or very dark brown.....32. *F. pallidefulva fuscata*
9. Clypeus with an anterior median notch (*sanguinea* group)10
 Clypeus unnotched13
10. Few or no hairs on dorsal surfaces of head and thorax.....11
 Erect hairs present on upper surfaces of head and thorax.....12
11. Dorsal surfaces of head infuscated, or the head at least darker than the thorax; both dark red21. *F. sanguinea aserva*
 Head not darker than the thorax; both lighter red....24. *F. sanguinea subnuda*
12. Gaster brown23. *F. sanguinea subintegra*
 Gaster black22. *F. sanguinea rubicunda*
13. Ground color of head and thorax red, although sometimes heavily infuscated; frontal area smooth and shining14
 Ground color of head and thorax black or at least not red; frontal area pubescent and rather opaque; hairs on gaster blunt (*fusca* group).....28
14. Head deeply excised behind (*ersecta* group)27
 Head at most feebly excised behind15
15. Petiole blunt, rather truncate or excised above.....16
 Upper border of petiole convexly or angularly produced, although sometimes with a notch in the middle17
16. Eyes hairy13. *F. reflexa*
 Eyes hairless12. *F. dakotensis montigena*

17. Erect hairs and pubescence nearly absent; gaster strongly shining. 10. *F. fossiceps*
Erect hairs on pubescence more numerous; integument more opaque.....18
18. Clypeal fossae deep; gaster rather sparsely pubescent, the surface not concealed.19
Clypeal fossae shallow; gaster often densely pubescent.....20
19. Smaller workers infuscated, majors and medium-sized workers with at least
the scale of the petiole infuscated9. *F. rufa clivia*
Smaller workers hardly darker than the majors, these with the petiole clear
red8. *F. rufa obscuriventris*
20. Erect hairs absent from dorsal surfaces of head and gaster.....11. *F. prociliata*
Erect hairs present on the dorsa of head and gaster21
21. Eyes hairy, erect hairs numerous22
Eyes hairless, erect hairs moderately abundant or sparse24
22. Erect hairs present on cheeks, oblique hairs on scapes.....14. *F. knighti*
No erect hairs on cheeks, no hair other than the pubescence on scapes; large
robust forms23
23. Pubescence dense on gaster, concealing the surface.....6. *F. rufa obscuripes*
Pubescence scarce on gaster7. *F. rufa melanotica*
24. Gaster rather shining, sparsely pubescent.....17. *F. nepticula*
Gaster opaque, densely pubescent25
25. Hairs apparently clavate; cheeks densely pubescent.....26
Hairs slender; cheeks sparsely pubescent18. *F. difficilis*
26. Hairs numerous, present on occipital corners of head...15. *F. microgyna spatulata*
Hairs sparse, not present on occipital corners.....16. *F. indianensis*
27. Front and vertex of head heavily infuscated; pronotum with numerous erect
hairs20. *F. ulkei*
Head not or scarcely infuscated; pronotum with no or very few hairs.....
.....19. *F. exsectoides*
28. Long erect hairs present on the gula.....5. *F. cinerea neocinerea*
Gula without erect hairs29
29. Thorax yellowish4. *F. fusca neoclara*
Thorax black30
30. Pubescence long and dense on all parts, giving a silvery appearance.....
.....3. *F. fusca argentea*
Pubescence shorter or less dense; body without a silvery appearance.....31
31. Pubescence dense on the gaster1. *F. fusca subsericea*
Pubescence on gaster rather sparse; body more shining..2. *F. fusca subaenescens*

1. *Formica (Formica) fusca subsericea* Say

- 1836 *F. subsericea* Say, Boston Jour. Nat. Hist., 1:289. ♂ ♀
 1913 *F. (Formica) fusca* var. *subsericea* Wheeler, Bull. Mus. Comp. Zool. Cambridge,
 53:398, 499. ♂ ♀ ♂
 1925 *F. (Serviformica) fusca subsericea* Emery, Gen. Insec., Fasc. 183:248.

Records: Ames, Clinton, Jewell, Spirit Lake, Backbone State Park, Carroll, Mt. Vernon, Oak Grove State Park. Also Sioux City (C. N. Ainslie).

This ant is common in all woodlands and in our cities and towns. It is the host of a number of parasitic *Formica*.

2. *Formica (Formica) fusca subaenescens* Emery

- 1893 *F. fusca* var. *subaenescens* Emery, Zool. Jahrb. Syst., 7:659. ♂
 1913 *F. (F.) fusca fusca* var. *subaenescens* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:399, 504. ♂ ♀ ♂

Record: Backbone State Park.

This variant is an inhabitant of deep woods where it nests in damp soil under rocks and logs. Apparently it is rare or absent in central Iowa where woodlands are rather scattered and usually somewhat open. *F.*

subaenescens may be the normal host of *Polyergus rufescens bicolor*, as will be shown in the discussion of the latter.

3. *Formica (Formica) fusca argentea* Wheeler

- 1902 *F. fusca* var. *argentata* Wheeler, Amer. Nat., 36:952 (in footnote). ♀ (nom. praeocc.)
 1912 *F. fusca* var. *argentea* Wheeler, Psyche, 19:90. (nom. nov.)
 1913. *F. (Formica) fusca fusca* var. *argentea* Wheeler, Bull. Mus. Comp. Zool., Cambridge, 53:398, 501. ♀ ♀ ♂
 53:398, 501. ♀ ♀ ♂
 1925 *F. (Serviformica) fusca subsericea* var. *argentea* Emery, Gen. Insec., fasc. 183:248.

Record: Stanhope (from prairie) (G. O. Hendrickson).

This species was probably a member of the original prairie fauna which has now been displaced in a large part by cultivation.

4. *Formica (Formica) fusca neoclara* Emery

- 1893 *F. fusca* var. *neoclara* Emery, Zool. Jahrb. Syst., 7:661. ♀
 1913 *F. (Formica) fusca fusca* var. *neoclara* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:398, 509. ♀ ♀ ♂
 1925 *F. (Serviformica) fusca subsericea* var. *neoclara* Emery, Gen. Insec., fasc. 183:248.

Record: Sioux City (C. N. Ainslie).

The validity of this record is somewhat doubtful as this variant is usually found only in the foothills of the Rocky Mountains. The writer suspects the specimens purported to be from Iowa may have been mislabeled.

5. *Formica (Formica) cinerea neocinerea* Wheeler

- 1902 *F. cinerea* Wheeler, Amer. Nat., 36:947.
 1910 *F. cinerea* var. *neocinerea* Wheeler, Ants, p. 571. ♀
 1913 *F. (Formica) cinerea cinerea* var. *neocinerea* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:399, 524. ♀ ♀ ♂
 1925 *F. (Serviformica) cinerea* var. *neocinerea* Emery, Gen. Insec., fasc. 183:246.

Records: Jewell, Ames, Spirit Lake.

This ant prefers to nest in the tops of boggy hummocks in pasture land, and probably could be found in any part of the state where such hummocks are present. It is more aggressive than the forms of *fusca*.

6. *Formica (Formica) rufa obscuripes* Forel

- 1886 *F. rufa* st. *obscuripes* Forel, Ann. Soc. Ent. Belg., 30 (C.R.):29. ♀
 1913 *F. (F.) rufa aggerans* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:392, 394, 430. ♀ ♀ ♂
 1940 *F. rufa obscuripes* Creighton, Amer. Mus. Nov., 1055:1, 7.

Records: Oak Grove State Park, Inwood, McGregor, Spencer. Also Ruthven (J. B. Low); Thompson (T. S. Baskett); Ocheyedon, Stanhope, Thompson, Westfield (G. O. Hendrickson).

This ant is often called the "thatching ant" because of the large

mound nest composed of twigs and other plant debris which these ants construct. All specimens from Iowa show more melanism, even in the largest workers, than is common in specimens of *obscuripes* from the Great Plains. Thus they may be considered transitional to the following variant, *melanotica*.

7. *Formica (Formica) rufa melanotica* Emery

- 1893 *F. rufa obscuriventris* var. *melanotica* Emery, Zool. Jahrb. Syst., 7:644, 650. ♀
 1913 *F. (F.) aggerans* var. *melanotica* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:392, 394, 432. ♀ ♀ ♂
 1940 *F. (F.) rufa melanotica* Creighton, Amer. Mus. Nov., 1055:1, 7.

Record: Denison.

The several nests of this variant near Denison were found in pasture land densely covered by scrub oaks so that all nests were shaded. This is in contradistinction to the nests of the form the writer has referred to *obscuripes*, whose nests were chiefly in virgin prairie, or at least exposed to the sun.

8. *Formica (Formica) rufa obscuriventris* Mayr

- 1870 *F. rufa obscuriventris* Mayr, Verh. Zool.-bot. Ges. Wien, 20:951. ♀
 1. 13 *F. (Formica) rufa obscuriventris* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:392, 394, 445. ♀ ♀ ♂

Records: Backbone State Park, Dubuque, Muscatine, Mt. Vernon.

This variant constructs its nests in old, dry, rotted stumps or logs, filling up the cavities with plant debris. It is very fierce and aggressive in the defense of its nest, like *obscuripes* and *melanotica*. In macroscopic aspect, color, size, aggressiveness, nesting habits, etc., this ant is almost identical with *F. sanguinea aserva*.

9. *Formica (Formica) rufa clivia* Creighton

- 1917 *F. (F.) rufa obscuriventris* var. *aggerans* Wheeler, Proc. Amer. Acad. Arts Sci., 52:540.
 1940 *F. (F.) rufa clivia* Creighton, Amer. Mus. Nov., 1055:8.

Records: Spirit Lake. Also Okoboji (F. S. Stancliffe).

The erect hairs of this variant seem rather deciduous. Therefore single workers are not easily identified. The nests found by the writer were under rocks banked with plant debris. This variant is apparently rare in Iowa, as it is more properly a member of Merriam's Transition Zone. The writer has not seen any specimens from Iowa which he considers intergrades between *clivia* and *obscuriventris*, although, according to Creighton (1940a), they occur in Minnesota.

10. *Formica (Formica) fossiceps* Buren

- 1942 *F. fossiceps* Buren, Iowa State Coll. Jour. Sci., 16(3):402-405. ♀ ♀ ♂

Type locality: Winterset.

The temporary host of this species is probably *F. fusca subsericea*.

11. *Formica (Formica) prociliata* Kennedy and Dennis1937 *F. prociliata* Kennedy and Dennis, Ann. Ent. Soc. Amer., 30:531. ♀ ♀ ♂

Records: Sabula, Bellevue, Winterset, Denison, Inwood.

This species lives in fairly populous colonies and constructs a low, flattened mound of earth about 2 or 3 feet in diameter. At Gotham, Wisconsin, the author found a female of *prociliata* which had been adopted by a depauperate colony of *F. (neofornica) pallidefulva nitidiventris*. *F. nitidiventris* may, therefore, be considered as the host or at least an alternate host of *prociliata*.

12. *Formica (Formica) dakotensis montigena* Wheeler1904 *F. montigena* Wheeler, Bull. Amer. Mus. Nat. Hist., 20:374. ♀ ♀ ♂1913 *F. (F.) dakotensis* var. *montigena* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:391, 394, 463. ♀ ♀ ♂

Record: Cherokee County (Prof. H. E. Jaques' Nat. Hist. Survey).

The writer has not taken specimens of this species from Iowa but has seen a specimen belonging to this form and collected in Iowa in the National Museum.

13. *Formica (Formica) reflexa* Buren1942 *F. reflexa* Buren, Iowa State Coll. Jour. Sci., 16(3):399-402. ♀ ♀ ♂

Record: Spirit Lake.

Several colonies of this interesting species were taken at the above locality. In each case the colony consisted of only a few *reflexa* workers but numerous workers of the host, *F. fusca subsericea*. As the writer has stated (1942), this species may possibly be a nondulotic, permanent social parasite. This type of parasitism has been hitherto unknown in the genus *Formica*.

14. *Formica (Formica) knighti* n. sp.

WORKER. Length of major worker, 7.5 mm.

Head, excluding mandibles, about as broad as long, with posterior border feebly excised in the middle, the posterior corners rounded, and sides slightly convex; scarcely narrower in front than behind. Mandibles 8-toothed. Clypeus rather angularly produced. Frontal area small, much wider than high. Frontal carinae evenly diverging, their length equal to twice the diameter of the antennal foramina. Eyes hairy. First funicular joint one-fourth again as long as the second, the second slightly longer than the third, and each joint to the penultimate in turn slightly longer than the succeeding, the second almost one-half again as long as the penultimate. Promesonotal outline strongly convex. Mesoepinotal impression deep and wide in large workers; marked by sutures before and behind. Declivity of epinotum a little longer than the base, meeting the latter with an angle of approximately 120-130 degrees. Petiole cuneate in profile, anterior and posterior faces weakly convex. Superior border

rather sharp; seen from behind angularly produced upward but usually notched at the tip.

All surfaces opaque except the frontal area, which is smooth and shining, and the mandibles, which are moderately shining and longitudinally striate.

Erect hairs numerous, short, bristle-like, yellow, usually pointed at the tip but on the thoracic dorsum and gaster sometimes blunt or slightly clavate. Hairs present on all regions, even a few on the cheeks; few, however, on the gula. The numerous hairs on the scapes and legs short and strongly oblique or subappressed. Pubescence dense in all regions.

Ground color of head and thorax yellowish red, but usually heavily infuscated with black, even in the largest workers. Smaller workers have the head and thorax nearly as black as the gaster.

Described from numerous workers taken from a single nest near Bonaparte, July 13, 1941. The nest was located in pasture land covered with a rather dense growth of scrub oaks. The nest was well hidden under low bushes, and considerable plant debris had been used in the construction of a low dome, immediately under which were numerous workers and the brood.

This species has about the same coloration as *F. postoculata* Kennedy and Dennis but does not seem closely related to it. *F. postoculata* has no hairs on the eyes, and no pilosity on the scapes or tibiae. It is much smaller in size, and there are several other differences in pilosity and in the shape of the head and thorax.

F. knighti appears most closely related to *F. impexa* Wheeler, which it strongly resembles in the number and arrangement of the hairs. *F. knighti* may be distinguished immediately from *impexa* by the color of the head and thorax, which is deep red in *impexa* and scarcely infuscated except in the smaller workers. The head of *impexa* is less robust, more slender, and narrower in front; the clypeus is less produced and is rounded in front; the thorax appears less robust, and the mesoepinotal constriction is shallow and narrow; the petiole is blunter and more rounded when seen from behind; the erect hairs are blunt or clavate, and the hairs on the scapes and legs are blunter and erect. The erect hairs on the gaster are more numerous and larger and more conspicuous in *impexa*. The pubescent hairs also seem a little denser but shorter on *impexa*. The eyes of *impexa* are not distinctly hairy as in the new species. *L. knighti*, incidentally, is one of the few microgynous species with hairy eyes.

Since the queen is unknown, there is no actual evidence that *knighti* is a microgynous species, but its general habitus and close resemblance to *impexa* lead the writer to believe so. It is certainly distinct from any species in the *rufa* group known to the author. *F. knighti* would perhaps key down to *F. oreas* Wheeler in Wheeler's key to the *Formica* (1913), but workers of *oreas* may be distinguished immediately by the extremely abundant, very fine white hairs covering all parts. Many other differences show that *oreas* is not closely related to *knighti*.

F. knighti is probably a temporary social parasite of *F. fusca subsericea*.

I take great pleasure in dedicating this species to Dr. H. H. Knight, Professor of Entomology, Iowa State College.

In the author's unpublished thesis, this species had a manuscript name.

15. *Formica (Formica) microgyna* subsp. *spatulata* n. subsp.

WORKER. Length of largest worker, 7.0 mm.

Head a little longer than broad, with slightly convex posterior border and sides, somewhat narrower in front than behind. Clypeus subangularly produced. Basal funicular joints longer than penultimate joints. Pro- and mesonotum moderately convex. Mesoepinotal impression shallow. Epinotum rounded, the declivity rather gently sloped. Petiole narrow, blunt, and angularly produced upward, the apex sometimes truncated or notched, however.

Nearly all surfaces opaque. Frontal area shining. Erect hairs short, spatulate, becoming very wide and flattened toward the apex; rather abundant on nearly all surfaces, including the occipital corners. Not present on scapes or tibiae. The tips of the hairs appear somewhat frayed under high power of the binoculars. Pubescence dense and fine on all parts, adding to the opaque appearance.

Head and thorax orange-red to brownish red, apparently depending on the age of the individual. Gaster black.

FEMALE. Length, 5.7 mm.

Head much smaller than in major worker, posterior border more rounded. Eyes a little smaller in absolute size, but larger and more convex in proportion to the head. Thorax narrower than the head, elongate. Epinotum rather sloping. Petiole much as in the worker.

Less opaque than in the worker. Erect hairs spatulate but much longer than in the worker; present on the same regions. Pubescence dense.

Ground color of head and thorax yellowish red, but these regions, especially the dorsal surfaces, rather infuscated. Gaster black. Wings pale.

MALE. Length, 7.0 mm.

Mandibles pointed, edentate. Thorax narrower than the head. Petiole blunt, not or only slightly notched. Erect hairs somewhat more abundant than in the female, a little shorter, and only feebly spatulate. Pubescence sparser than in the female. Color black, with tibiae and tarsi yellowish. Wings pale hyaline.

Two nests of this form were found under rocks along the shore of a small lake near Spirit Lake, Iowa (type locality). Also included in the type series are some specimens (workers and females) from Wheaton, Minnesota.

This ant seems to be another geographical race of the widely distributed *Formica microgyna* Wheeler. *Spatulata* is more slender-bodied than most of these variants, the promesonotal outline is less convex, and the epinotum is more obtuse. The hairs are quite short and are more widened and flattened toward the apex than in any of the *microgyna* variants seen by the author.

From typical *microgyna*, *spatulata* may be easily distinguished by the lack of erect hairs on the scapes and the more spatulate hairs on the body, as well as the more slender body, less convex promesonotum, and more obtuse epinotum. From *microgyna rasilis*, with which it is perhaps most closely related, *spatulata* may be distinguished by the more numerous erect hairs and their presence on the occipital corners of the head, as well as the body proportions mentioned above. The same differences will also apply to *F. querquetulana* Kennedy and Dennis.

The female of *spatulata* is somewhat more slender-bodied than females of *microgyna*, *microgyna rasilis*, and *querquetulana*, and the same differences in pilosity mentioned for the workers are applicable. The petiole of the *spatulata* female is narrower than that of the *querquetulana* female.

F. microgyna spatulata may be distinguished immediately from all other Iowa species of *Formica* by the beautiful, dull, orange-red color of the head and thorax, which is especially striking in the younger workers.

The temporary host is *Formica fusca subsericea* Say, specimens of which were found in one of the Spirit Lake nests.

16. *Formica (Formica) indianensis* Cole

1940 *F. indianensis* Cole, Amer. Midl. Nat. 23:224-226. ♀ ♂

Record: Oak Grove State Park.

The author has taken 24 workers from at least two nests on a virgin prairie remnant at Oak Grove State Park. Stray workers were picked up, but the actual nests were not found and must have been small and well hidden.

The writer has taken a series of workers from a single nest at Inwood, Iowa, which shows all possible intergradations with *F. nepticula*. *F. indianensis* must therefore be closely related to *nepticula* in spite of their dissimilar appearance.

F. indianensis is probably a temporary social parasite of *F. fusca subsericea* or possibly *F. fusca argentea*.

17. *Formica (Formica) nepticula* Wheeler

1905 *F. nepticula* Wheeler, Bull. Amer. Mus. Nat. Hist., 21:270. ♀ ♀ ♂

1913 *F. (F.) nepticula* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:394, 396, 475. ♀ ♀ ♂

Record: Denison.

The nests of this species are usually located in and under small rotting limbs; some plant debris is used. Winged females were found in a nest in July.

18. *Formica (Formica) difficilis* Emery

1893 *F. rufa difficilis* Emery, Zool. Jahrb. Syst., 7:651. ♀ ♀ ♂

1913 *F. (F.) difficilis* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:395, 477. ♀ ♀ ♂

Records: Boone, Ames, Bellevue.

On a smaller scale, the nest architecture is like that of *F. rufa obscuriventris*. *F. difficilis* is more timid and less aggressive than many ants of the *rufa* group. Its host is undoubtedly some form of *F. pallidefulva* Latreille.

19. *Formica (Formica) exsectoides* Forel

- 1886 *F. exsectoides* Forel, Ann. Soc. Ent. Belg., 30(C.R.):38. ♀ ♀
 1913 *F. (F.) exsectoides exsectoides* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:396, 481. ♀ ♀ ♂

Records: Inwood, Denison, Mt. Vernon.

This species does not seem to thrive well in Iowa. The mounds that the writer has seen were rather small and scarcely conical. *F. exsectoides* often lives in huge aggregate colonies consisting of numerous mounds. In Iowa the writer has been unable to find more than a single mound in any one locality.

20. *Formica (Formica) ulkei* Emery

- 1893 *F. ulkei* Emery, Zool. Jahrb. Syst., 7:653. ♀
 1913 *F. (F.) ulkei* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:396, 485. ♀ ♀ ♂

Record: Spirit Lake.

F. ulkei is apparently not common in any part of its range, and in Iowa must be very rare even in the northern part. The colony found by the writer was rather depauperate.

21. *Formica (Formica) sanguinea aserva* Forel

- 1901 *F. sanguinea aserva* Forel, Ann. Soc. Ent. Belg., 45: 395. ♀ ♀
 1913 *F. (Formica) sanguinea aserva* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:389, 404. ♀ ♀ ♂
 1925 *F. (Raptiformica) sanguinea aserva* Emery, Gen. Insec., fasc. 183:230.

Record: Rice Lake State Park.

This form has a rather boreal distribution. The writer has found it to be common in Minnesota and Wisconsin, but it seems rare even in the northern portions of Iowa. This ant is very fierce and aggressive but does not have dulotic habits. Its favorite nesting sites are old rotting stumps, a certain amount of plant debris being used around the base and in the large cavities.

22. *Formica (Formica) sanguinea rubicunda* Emery

- 1893 *F. sanguinea rubicunda* Emery, Zool. Jahrb. Syst., 7:647. ♀ ♀
 1913 *F. (Formica) sanguinea rubicunda* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:390, 406. ♀ ♀ ♂
 1925 *F. (Raptiformica) sanguinea rubicunda* Emery, Gen. Insec., fasc. 183:230.

Records: Dennison, Ames, Sabula, Oak Grove State Park, Clinton.

This ant is much more common in woodlands than *subintegra* but does not live in cities or towns. This is another example of how civilization has changed the fauna, reducing the numbers of some species, increasing those of others.

23. *Formica (Formica) sanguinea subintegra* Emery

- 1893 *F. sanguinea rubicunda* var. *subintegra* Emery, Zool. Jahrb. Syst., 7:648. ♀ ?
 1913 *F. (Formica) sanguinea subintegra* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:390, 410. ♀ ♀ ♂
 1925 *F. (Raptiformica) sanguinea subintegra* Emery, Gen. Insec., fasc. 183:260.

Records: Ames, DeWitt.

This form seems to thrive well in lawns in cities and towns, unlike *rubicunda*, which is never found in such a situation. It is common within Ames, and the writer has also seen it at Clinton.

24. *Formica (Formica) sanguinea subnuda* Emery

- 1895 *F. sanguinea rubicunda* var. *subnuda* Emery, Zool. Jahrb. Syst., 8:335. ♀
 1913 *F. (Formica) sanguinea subnuda* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:389, 409. ♀ ♀ ♂
 1925 *F. (Raptiformica) sanguinea subnuda* Emery, Gen. Insec., fasc. 183:260.

Record: Sioux City (C. N. Ainslie).

Represented by six specimens found in the collection of the late C. N. Ainslie.

The epinotum is angulate in these specimens as in the preceding forms. They do not agree in this particular with Emery's description of *subnuda* (1894), and therefore may not actually be *subnuda*. For the present the writer prefers to regard them as such. The pilosity is the same as that of *aserva*, but *aserva* has a much darker colored and broader head.

25. *Formica (Proformica) neogagates* Emery

- 1893 *F. fusca subpolita* var. *neogagates* Emery, Zool. Jahrb. Syst., 7:661. ♀ ♀ ♂
 1913 *F. (P.) neogagates neogagates* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 536. ♀ ♀ ♂

Records: Ames, Tama, Spirit Lake.

A rather rare species. Only two small nests have been found under stones near Ames. The Tama and Spirit Lake records are from single specimens whose nests could not be located.

The specimens listed as *neogagates* in the writer's preliminary list (1941a) are *neogagates vinculans*.

26. *Formica (Proformica) neogagates vetula* Wheeler

- 1895 *F. lasioides* var. *picea* Emery, Zool. Jahrb. Syst., 8:335. ♀ (nom. praeocc.)
 1912 *F. (P.) neogagates lasioides* var. *vetula* Wheeler, Psyche, 19:90. (nom. nov.)
 1913 *F. (P.) neogagates lasioides* var. *vetula* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 540. ♀

Records: Ames, Rice Lake State Park, Strawberry Point, Decorah, Inwood.

This ant seems to be the commonest form of *neogagates* in Iowa. It lives in small colonies in woodlands.

27. *Formica* (*Proformica*) *neogagates vinculans* Wheeler

- 1913 *F. (P.) neogagates* var. *vinculans* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 539. ♀ ♀

Records: Ames, Inwood.

This ant is rather common in lawns in Ames, a situation where the typical *neogagates* does not occur. The nests are rather small but more populous than the nests of typical *neogagates* seen. The ants will swarm out to defend their nests if provoked.

28. *Formica* (*Proformica*) *neogagates morbida* Wheeler

- 1913 *F. (P.) neogagates* var. *morbida* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 538. ♀ ♀

Type locality: Lennox (P. J. Schmitt).

The writer does not possess specimens of this form but has seen the types in the Museum of Comparative Zoology at Cambridge, Mass.

29. *Formica* (*Neoformica*) *pallidefulva incerta* Emery

- 1893 *F. pallidefulva schaufussi* var. *incerta* Emery, Zool. Jahrb. Syst., 7:655. ♀ ♀ ♂
 1913 *F. (N.) pallidefulva schaufussi* var. *incerta* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 554. ♀ ♀ ♂

Records: Ames, Clinton, Tama, Holy Cross, Rice Lake State Park, Inwood.

A common form in Iowa. There is considerable variation in color in the Iowa specimens. Some are as light as *pallidefulva schaufussi* Mayr, others as dark as *pallidefulva nitidiventris*. Nevertheless, the Iowa specimens almost always have the erect hairs lacking on the gula, and so all have been referred to *incerta*. The writer is convinced that *nitidiventris* can always be distinguished from *incerta* by its lack of both gular and petiolar hairs, and by its shorter, blunter gastric hairs, in spite of the frequent convergence in color.

30. *Formica* (*Neoformica*) *pallidefulva dolosa* Wheeler

- 1904 *F. pallidefulva schaufussi* var. *meridionalis* Wheeler, Bull. Amer. Mus. Nat. Hist., 20:370. ♀ (nom. praeocc.)
 1912 *F. pallidefulva schaufussi* var. *dolosa* Wheeler, Psyche, 19:90. (nom. nov.)
 1913 *F. (N.) pallidefulva schaufussi* var. *dolosa* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 554. ♀ ♀

Record: Glenwood.

This is a southern variant which apparently has managed to creep its way northward into Iowa only along the Missouri River bluffs.

F. pallidefulva dolosa is the only *Formica* which was found living on these bluffs. This is what one would expect if the Missouri River bluffs really have a southern fauna as has been contended. The genus *Formica* is poorly represented in the South.

31. *Formica (Neoformica) pallidefulva nitidiventris* Emery1893 *F. pallidefulva nitidiventris* Emery, Zool. Jahrb. Syst., 7:656. ♀ ♂1913 *F. (N.) pallidefulva nitidiventris* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 555. ♀ ♂

Records: Ames, Sabula, Oak Grove State Park, Princeton.

A common woodland form.

32. *Formica (Neoformica) pallidefulva fuscata* Emery1893 *F. pallidefulva* var. *fuscata* Emery, Zool. Jahrb. Syst., 7:656. ♀ ♂1913 *F. (N.) pallidefulva nitidiventris* var. *fuscata* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 557. ♀ ♀

Records: Ames, Clinton, Sabula, Holy Cross.

This form may have no validity other than as a mere color variety of *nitidiventris*. The females can scarcely be separated.7. *Polyergus*

KEY TO SPECIES OF POLYERGUS

- | | |
|---|----------------------------------|
| 1. Gaster pubescent | 2 |
| Gaster smooth and shining; pubescence very sparse | 1. <i>P. lucidus</i> |
| 2. Gaster red like the head and thorax | 2. <i>P. rufescens breviceps</i> |
| Gaster black | 3. <i>P. rufescens bicolor</i> |

1. *Polyergus lucidus* Mayr1870 *P. lucidus* Mayr, Verh. Zool.-bot. Ges. Wien, 20:952. ♀ ♀ ♂

Record: Backbone State Park.

This species probably has its western limit in Iowa. The slave of the colony found at Backbone State Park was *F. (Neoformica) pallidefulva incerta*. The ants listed as *lucidus* in the writer's preliminary list are *rufescens breviceps*.

2. *Polyergus rufescens breviceps* Emery1893 *P. rufescens breviceps* Emery, Zool. Jahrb. Syst., 7:666. ♀

Records: Ames. Also Sioux City (C. N. Ainslie).

This ant is fairly common in lawns in Ames, and the writer has seen it also within Clinton, Des Moines, and Davenport. It does not seem to occur, or at least must be very rare, outside city limits. In this peculiar preference it parallels *Formica sanguinea subintegra*.

3. *Polyergus rufescens bicolor* Wasmann1901 *P. rufescens bicolor* Wasmann, Allg. Zeitschr. f. Ent. Neudamm, 6(N):23. ♀ ♀ ♂

Record: Backbone State Park.

A single female with a swollen gaster was taken at Backbone State

Park. She had been adopted by a medium-sized nest of *Formica fusca subaenescens*. Colonies found by the writer at Akeley and Jenkins, Minnesota, also had the same species as the slave.

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THE SEASONAL HISTORY AND HOSTS OF THE AMERICAN DOG TICK, *DERMACENTOR VARIABILIS*, IN IOWA¹

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Biological studies on the American dog tick were started at the Tama Indian Reservation, Tama, Iowa, in April, 1941, and terminated in December; supplementary collecting was done at Ames during January, February, and March, 1942. This paper presents a summary of the data obtained.

Biological data concerning this tick were reported by Morgan (1899), Hunter and Hooker (1907), Banks (1908), Hooker (1908, 1909), and Hooker, Bishopp, and Wood (1912). These last authors recorded the adult ticks as occurring on 19 species of animals. They collected no larvae known to be of this species but found nymphs on the fox squirrel and swamp rabbit. Hadwen (1913) published data on the life history, and Hewitt (1915) gave an account of Canadian ticks but added little to Hadwen's information concerning *D. variabilis*.

The white-footed woodmouse, *Peromyscus leucopus*, was shown by Larrousse, King, and Wolbach (1928) to serve as a host for immature forms. Further information on taxonomy, distribution, seasonal history, and hosts was furnished by Cooley (1932, 1938). Bishopp and Smith (1938) published the most complete report, especially on the host relationship of the immature forms; and additional information on seasonal history and hosts was supplied by MacCreary (1940-1941).

This tick was recognized as a species for about 90 years before it was incriminated as a vector of human diseases. With it, Maver (1911) experimentally transmitted the western strain of Rocky Mountain spotted fever, and Dyer, Badger, and Rumreich (1931) showed it to be a vector of the eastern strain. Naturally infected specimens of the tick were collected in Virginia by Badger (1932). The tularemia organism was recovered from naturally infected ticks by Green (1931), and survival of this bacterium through stage-to-stage and generation-to-generation development of ticks was shown by Philip and Jollison (1934). The American dog tick is now considered to be the principal vector of Rocky Mountain spotted fever in the eastern United States. It was also incriminated in the transmission of bovine anaplasmosis by Rees (1932).

According to Jordan (1937), an outbreak of spotted fever occurred among the Indians at the Sac and Fox Reservation, Tama, Iowa, begin-

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ning in 1933; the prevalence of ticks in the area has been reported several times. Studies on *D. variabilis* were undertaken on the Reservation, and were restricted to this locality.

The Tama Indian Reservation is composed of about 3,300 acres. In 1936 the total cordwood was 4,000,000 feet b.m., made up of soft maple, cottonwood, red and white elm, mixed oaks, basswood, walnut, hickory, boxelder, hackberry, honey locust, and ash, listed in the order of their timber volume. Parts of the area were in plantations of black ash (18.4 acres), Norway and white pine (45.2), and soft maple, cedar and walnut (4.9). Important cover for rodents and other wildlife was formed by clumps of trees and shrubs found mostly in sparsely covered areas. In general, the Reservation varies from a hilly, sparsely forested, and well-drained condition in the north, to a flat, densely covered, and semi-swampy condition where the Iowa river traverses it in the southern portion.

The dog serves as the principal host for the adult stage of *D. variabilis*. According to Bishopp and Smith (1938), the female requires from 5 to 13 days for engorgement. Although no experiments were undertaken concerning this, general observations on engorgement periods support these findings. Bi-weekly examinations of dogs were made in order to assure the removal of ticks before repletion and to obtain seasonal history data. Occasionally a replete specimen was found, but it was considered to have been missed at the previous examination. Some of the dogs were checked for a short period and then disappeared, but a few were available for examination throughout the entire course of the investigation. Dogs that were "deticked" periodically throughout the spring, summer, and fall presented very interesting individual records.

No special effort was made to examine larger domestic animals during the active tick season. There were a number of horses on the Reservation, but few cattle. These domestic animals were examined closely during September, October, and November, or when ticks became scarce.

Field mice are considered the most important hosts for the immature forms and for this reason were live-trapped for seasonal tick activity and host records. A single-catch metal type of trap with a length of 5½ inches and a width of 4 inches was used. A small wad of cotton was placed in each trap as nesting material and for aid in detection of engorged ticks dropped from the mice. Metal traps are known to cause a high mortality of mice from heat during the summer and by low temperatures in the winter. However, little mortality will occur during the summer months if the traps are kept in the shade; during the colder months it is difficult to control this factor when metal traps are used. In order to catch both diurnal and nocturnal animals, the traps were kept set both day and night. The northern white-footed mouse is nocturnal, whereas the literature concerning the activity of species of the genus *Microtus* is conflicting. The latter are said to forage chiefly during the day, but their activity probably depends to some extent on the amount of cover present. That the type of trap used was suitable for *Peromyscus* was evidenced by the re-

capture of a large percentage of the mice, some of them a number of times, depending upon the length of time the traps remained in the same area. Whether the trapping method was satisfactory for the other species present is not known; some species are no doubt more difficult to trap than others. Mice were deticked in the field, ear-tagged, and released at the point of capture. The traps were set from 2 to 4 rods apart, either in a grid pattern or in a straight line, according to the vegetation present. When the ticks or mice became scarce in one area the traps were usually moved a distance greater than the known range of the mice.

Other animals such as cottontail rabbits, woodchucks, ground squirrels, and birds were shot, bagged, and taken to the laboratory for examination.

DISTRIBUTION

The American dog tick is reported to be common in certain parts of southern Canada. It is known to occur throughout the United States east of the Rocky Mountains, and is prevalent in parts of California. In the southern and eastern half of Iowa, this tick is well represented, according to the Iowa State Department of Health reports. Although apparently more numerous in these areas, it is no doubt present in other suitable habitats of the state. Hosts for the adult and immature stages are known to occur throughout Iowa.

ADULT ACTIVITY

Adult specimens have been collected on animals in Texas at all times of the year, according to Hooker, Bishopp, and Wood (1912). Hadwen (1913) states: "As soon as the snow disappears and the warm weather begins, adults are apparently found everywhere." Cooley (1932) mentions the inactivity of the adults during the winter months in the North. Bishopp and Smith (1938) report that in Maryland and adjacent states the ticks begin to appear from the middle of March to the middle of April, depending upon the temperature. They also state that few ticks are seen after August 1. Specimens have been collected in Oklahoma by the senior author as early as March and as late as September. Though no extensive search was made during the colder months, the adults are probably inactive during the winter period in that state. The adults are found on dogs in Delaware from April 29 through August 23, according to MacCreary (1941).

Several attempts by means of the "drag" or "flag" method were made at Ames, Iowa, to collect adults. No specimens were taken by this method. One male was taken on April 8, and a male and female on April 12, from debris on the ground. No dogs had been examined in areas where ticks were known to occur before the above dates. Six males and two females were removed from two dogs examined on April 16, at the Indian Reservation at Tama. One of the females was about three-fourths replete. These records seem to indicate that the adults of *D. variabilis* were active during the first week in April, but perhaps to a limited extent. Bi-weekly

examinations of dogs were made thereafter in an attempt to obtain seasonal history data only. No effort was made to collect large numbers of ticks, but certain dogs were deticked regularly and thoroughly. As mentioned above, some of the dogs were checked throughout the course of the work. It was thought that a decrease in the population of ticks might occur if specimens were removed from the dogs over such a period of time. However, this would probably be true only if the animals remained at home. Most of the dogs "strayed" from home either during the day or at night. Dogs that ran at large were more heavily infested than those remaining at home. To avoid undue local reductions in tick populations, certain dogs were left untagged and were never deticked. Those animals were included in examinations made in August, September, and November. They also served the purpose of increasing the number of examinations when ticks became scarce.

A summary by months of the number of ticks collected from dogs is given in Table 1. The average number per dog and the percentage of dogs infested are given. Since the work started on April 16, only 13 animals were examined during that month. The average number of ticks per dog increased from 40.53 in April to a peak of 89.17 in May, with a

TABLE 1

A MONTHLY AVERAGE OF TICKS (*D. variabilis*) COLLECTED FROM DOGS, APRIL 16, THROUGH DECEMBER 21, 1941, AND THE PERCENTAGE INFESTED

Month	No. Dogs Examined	Total No. of Ticks	Adult Ticks		Average No. of Ticks	Percentage of Dogs Infested
			Males	Females		
April.....	13	527	327	200	40.53	100
May.....	68	6,064	3,139	2,922	89.17	100
June.....	111	3,138	1,631	1,483	28.27	100
July.....	151	1,582	759	807	10.47	94.0
August.....	118	104	37	61	.88	41.5
September.....	251	26	11	12	.10	6.4
October.....	248	1	1	0	.004	.4
November.....	116	0	0	0	0	0
December.....	56	0	0	0	0	0
Totals.....	1,132	11,442	5,905	5,485		
Mean average.....					(10.10)	(49.14)

marked decrease for the months thereafter. The last replete female was taken on September 18. This specimen was noted at the previous examination and was left for engorgement. Only one male specimen was removed (Oct. 9) from the 248 animals checked in October. No ticks were found on animals examined in November or December. For the season, a total of 5,905 males and 5,485 females were taken. The females were more active during the rapid population decline of July, August, and September. A very interesting "natural" decrease of ticks was noted when the average number per dog fell from 89.17 in May to 28.27 in June, but the percentage infested remained the same. A few nymphs and

larvae were collected from dogs; these will be discussed below in the section which treats of those stages. Adult ticks were removed from the following animals: cow, horse, pig, raccoon, fox squirrel, house cat, and woodchuck.

Concerning hosts of the immature stages, very little has been published. Hunter and Hooker (1907) failed in an attempt to feed larvae on cattle. Hunter and Bishopp (1910) state that the young stages are found upon various small mammals, but mention no particular species. Nymphs were reported collected from the fox squirrel and swamp rabbit by Hooker, Bishopp, and Wood (1912). They induced larvae to attach to a bovine, but did not succeed in feeding that stage on dogs. The white-footed woodmouse, *Peromyscus leucopus*, was reported as a host for the larvae and nymphs by Larrouse, King, and Wolbach (1928). The most important data concerning hosts of immature stages were published by Bishopp and Smith (1938). Their work is quoted because of its importance: "The following is a list of the collections of immature stages contained in the accession catalogue. The number of times nymphs and larvae have been taken on the respective hosts is as follows: whitefooted mice (*Peromyscus*): larvae 68, nymphs 18; meadow mice (*Microtus*): 12, 13; pine mice (*Pitymys*): 4, 8; house mouse (*Mus domesticus*): 3, 0; kangaroo mouse (*Zapus*): 1, 1; mouse, species in doubt: 5, 5; cottontail rabbit: 3, 8; swamp rabbit: 2, 1; cotton rat (*Sigmodon hispidus*): 3, 1; Norway rat: 2, 1; wood rat (*Neotoma*): 0, 1; squirrels: 0, 7; cat: 0, 2; shrew (*Blarina brevicauda*): 2, 1; sheep: 0, 1 (unengorged); cattle: 0, 1 engorged; mole (*Scalopus aquaticus machrinus*): 1 unengorged, 0. The larger number of collections of ticks from *Peromyscus* as compared with those from *Microtus* was due to the much larger number of *Peromyscus* collected." MacCreary (1940) states that the meadow mouse is the preferred host of the spotted fever tick in Delaware. Similar observations were made by the same author the following year, and he found a number of other mice infested as well. Larval and nymphal stages were taken from the domestic rat and cottontail rabbit, but no specimens were found on the few birds he examined.

The average numbers of larval and nymphal ticks collected from the northern white-footed mouse are presented below in Table 2. This species was greatly predominant among mice on the Reservation. More than 95 per cent (2,656) of the mice trapped were of this species. The increase in the number caught, from 286 in July to 496 in August, was probably due in part to an increase in the number of traps employed. Since the work was started on April 16, the early spring emergence of the young ticks was not ascertained in 1941. Several of the mice trapped in April were infested with more than 100 larvae, one with 186. Some ticks were probably lost from the latter mouse, since it was examined several hours after death. The average of 32 larvae per mouse in April far exceeded that of any other month. The number gradually decreased from 3.36 in May to .01 in November. The last larva was taken on November 23. No specimens were taken from the 238 mice examined in December, January, and February. The mice trapped in January, February, and March

TABLE 2

A MONTHLY AVERAGE OF LARVAE AND NYMPHS (*D. variabilis*) COLLECTED FROM THE NORTHERN WHITE-FOOTED MOUSE, *Peromyscus leucopus noveboracensis*, FROM APRIL 16, 1941, THROUGH MARCH 31, 1942, AND THE PERCENTAGE OF MICE INFESTED

Month	No. Mice Examined	Total No. of Ticks	Average No. per Mouse		Percentage of Mice Infested
			Larvae	Nymphs	
April, 1941.....	40	1,290	32.05	.20	90.00
May, 1941.....	149	609	3.36	.72	78.53
June, 1941.....	168	425	1.25	1.27	69.04
July, 1941.....	268	594	1.09	.98	60.48
August, 1941.....	496	1,170	1.99	.36	56.04
September, 1941..	490	425	.73	.11	39.59
October, 1941.....	450	124	.24	.02	18.00
November, 1941...	299	6	.01	.007	1.67
December, 1941..	191	0	0	0	0
January, 1941....	17	0	0	0	0
February, 1942....	30	0	0	0	0
March, 1942.....	40	15	.35	.02	.12
Totals.....	2,638	4,658			

were taken at Ames, Iowa. The first larvae collected in the spring of 1942 were taken on March 24. Five of the six mice examined thereafter were infested. Even though replete nymphs were collected on April 17, the average was far below that of the larvae during April and May, 1941. They were slightly more numerous in June, but were less active during the latter part of the summer and fall. The last specimen was collected on November 8. As with the larvae, no specimens were taken in December, January, and February. The first nymph collected in the spring of 1942 was taken on March 31. The immature stages collected in March were taken from areas where trapping had been done during January and February. It is likely that inactive larvae and nymphs were present in those areas during January and February.

It can be seen from the above table and by records from the cottontail rabbit (Table 3) that the larvae were more active in April than at any other time. Few nymphs were present during April, but they increased to a peak in June. This was evident not only on *Peromyscus*, but on practically all the other animals listed in Table 3. That the immature forms are inactive during the winter months is indicated by the number of mice examined with negative results during that period, and by the fact that mice examined in the same areas in March were infested. Bishopp and Smith (1938) collected larvae and nymphs on field mice in the District of Columbia and nearby Maryland and Virginia during the winter months. They also state that seed ticks were relatively scarce in the spring, but nymphs were present in large numbers.

The number of larval and nymphal ticks collected from some of the other animals is presented below in Table 3. Some of the data are insignificant but are given, since more information is needed concerning hosts of immature stages.

TABLE 3
NUMBERS OF LARVAL AND NYMPHAL TICKS (*D. variabilis*) COLLECTED FROM VARIOUS ANIMALS, APRIL 16, THROUGH DECEMBER 21, 1941

Month	Meadow Mouse, <i>Microtus p. pennsylvanicus</i>			Prairie Harvest Mouse, <i>Reithrodontomys megalaotis dychei</i>			Fox Squirrel, <i>Sciurus niger rufiventris</i>			Rabbit, <i>Sylvilagus floridanus meansi</i>			House Cat, <i>Felis domestica</i>			Dog, <i>Canis familiaris</i>		
	No. Exam.	L*	N*	No. Exam.	L	N	No. Exam.	L	N	No. Exam.	L	N	No. Exam.	L	N	No. Exam.	L	N
April.....	0	0	0	0	0	0	0	0	0	13	249	2	0	0	0	13	0	0
May.....	4	38	4	1	0	0	8	2	0	10	68	9	6	151	2	68	1	2
June.....	6	3	23	2	0	0	4	3	7	5	2	36	7	23	2	111	1	23
July.....	0	0	0	2	0	0	4	0	0	4	2	0	4	7	3	151	1	15
August.....	1	21	0	10	4	1	3	1	1	5	26	5	4	7	1	118	4	2
September.....	0	0	0	26	1	0	3	0	0	5	2	0	1	0	1	251	3	0
October.....	0	0	0	34	2	0	1	0	0	2	0	0	0	0	0	248	0	0
November.....	0	0	0	11	0	0	1	0	0	6	0	0	0	0	0	116	0	0
December.....	6	0	0	6	0	0	0	0	0	6	0	0	0	0	0	56	0	0
Totals.....	17	62	27	92	7	1	24	6	8	56	349	52	22	188	8	1132	10	42

* L = Larvae; N = Nymphs.

Four records obtained are not included in Table 3. A nymph was taken from the woodchuck, *Marmota m. monax*, in May, and three flat larvae were removed from the Virginia opossum *Didelphis v. virginiana*, on August 31. One larva was removed from the house mouse, *Mus. m. musculus*, in August, and one larva was taken in July from the prairie jumping mouse, *Zapus hudsonius campestris*. Eleven woodchucks, two opossums, one house mouse, and two prairie jumping mice were examined.

Meadow mice were either difficult to trap or were present only in small numbers. The 17 animals examined yielded 27 nymphs and 62 larvae. However, six of these mice were trapped in December when no ticks were active. The records tend to indicate the abundance and seasonal trend of the immature ticks. If the number trapped is an index of the number present, meadow mice probably are not of great importance as hosts on the Reservation.

The prairie harvest mouse is even less important than the above species. One nymph and seven larvae were collected from the 92 trapped mice. Because of small amount and short length of hair this mouse affords little protection to the ticks that do attach.

Eight nymphs and six larvae were removed from the 24 fox squirrels examined. Nymphs have been collected from fox squirrels by different workers, but Bishopp and Smith (1938) record no larvae.

The larvae were more active in April as evidenced by the number of specimens collected from the cottontail rabbit. The seasonal trend of both larval and nymphal stages on the cottontail is similar to that on the northern white-footed mouse and in part, that on other animals. The 56 animals examined yielded 52 nymphs and 349 larvae. One rabbit shot on the laboratory grounds was infested with engorging immature ticks. It appears that the cottontail rabbit, at least under certain conditions, might be a more important host than is generally thought.

There were a number of house cats on the Reservation, but these were difficult to examine. It is unfortunate that at least a few were not checked in April, since 151 larvae were found on six cats during May. One of these was infested with 97 larvae. A total of 8 nymphs and 188 larvae were removed from the 22 cats examined. Bishopp and Smith (1938) record no larvae from the house cat.

The writers found in the literature no previous larval or nymphal records from the dog. A total of 10 larvae and 42 nymphs, representing 43 infestations, were collected, however, from 1,132 dogs examined at Tama, Iowa. The ticks were in all stages of development, many of the nymphs fully engorged. The engorged specimens had probably been overlooked at the previous examination. Flat specimens are difficult to find, especially larvae, and no doubt many were overlooked. A thorough examination in search of young ticks could not be given each dog, because of the amount of time involved.

Parker (1937) states that in *D. variabilis* territory there is a higher proportion of spotted fever cases in women and children than in men because of the close association of this tick with the home through the

agency of dogs. It would be interesting to know just how many cases of this disease have been the result of immature ticks completing their development on rabbits, house cats, dogs, or other animals that range near the home. There is little doubt that the American dog tick can complete its entire life-cycle in the "backyard."

PLACES OF ATTACHMENT

It is known that some ticks are not only specific for certain animals, but are specific as to the parts they parasitize. The areas of attachment may afford complete or incomplete protection for the tick.

No definite data were obtained on places of attachment for the adult stage of the dog tick; no doubt the greatest numbers attach about the head, neck, and shoulders, and along the back.

Some figures were obtained on the attachment of the larval and nymphal forms. Those data perhaps are not of great scientific value, but are of interest. They represent observations made from July through December. The percentages of larvae and nymphs infesting the various parts of the body are as follows: Larvae: ears, 71.07; cheeks, 10.08; other parts of the head, 8.49; back and shoulders, 5.74; neck, 3.60; legs, .73; sides and belly, .27; Nymphs: back and shoulders, 36.80; neck, 22.67; head (except ears and cheeks), 19.70; cheeks, 13.38; ears, 6.69; sides, .74. In the case of larvae, almost 90 per cent of the ticks were removed from the head, and 71 per cent of the total were found on the ears. Few specimens were found on the posterior part of the body. Infestations of the tail were noted several times in April when the ticks were numerous, but not after that date. In the case of nymphs, only about 40 per cent were found on the head and 6 per cent on the ears. Almost 60 per cent were found on the neck and along the forepart of the back. In general, it may be said that the larvae show a preference for the head, especially the ears, and that the nymphs tend to congregate about the neck and shoulders and forepart of the back.

SUMMARY

Seasonal history and host studies on the American dog tick, *Dermacentor variabilis*, were conducted at the Tama Indian Reservation, Tama, Iowa. The work started in April, 1941, and was terminated in December. Some collecting was done at Ames during January, February, and March to determine the spring emergence of the immature stages.

Bi-weekly examinations of dogs were made to determine the seasonal trend of adults, and mice were live-trapped for data on the seasonal history of young ticks. Other animals were checked at every opportunity.

Results of the "drag" and "flag" method at Ames and the collecting done at Tama, indicate the adult ticks began activity the first week in April. The average number of adult ticks per dog each month was approximately 40, 89, 28, 10, .8, .1, and .004, from April through October, respectively. The last specimen was taken on October 9. One hundred per cent of the dogs were infested during April, May, and June. A total

of 1,132 dogs were examined. No new host records for adults were obtained, but a number were collected from the horse, cow, pig, fox squirrel, woodchuck, house cat, and raccoon.

The work was started too late to determine the spring emergence of the larvae and nymphs in April, 1941. The average number of larvae removed from the northern white-footed mouse, *Peromyscus leucopus novoboracensis*, was approximately 32, 3, 1, 1, 1, .7, .2, and .01, and that of nymphs was .2, .7, 1.2, .9, .3, .1, .02, and .007, in the months of April through November, respectively. The last nymph was collected on November 8, and the last larva on November 23. In January and February, 1942, 47 mice were examined at Ames, but no ticks were found.

The 40 mice checked in March yielded 14 larvae and 1 nymph. The first larva was collected on March 24 and the first nymph on March 31. The ticks collected in March were taken from the same area where trapping had been done in January and February. These results seem to indicate that the immature stages are not active during the winter months in Iowa. A total of 2,656 white-footed mice, 92 prairie harvest mice, 19 meadow mice, 2 prairie jumping mice, and 1 house mouse were examined. The northern white-footed mouse appears to be the most important cricetine on the area. Results indicate that the prairie harvest mouse is not a very favorable host. Data on the other three species were too incomplete to warrant conclusions.

Larvae and nymphs were also collected from several other hosts. The total number of animals examined and the number of larvae and nymphs removed are as follows: dogs, 1,132 examined, 10 larvae and 42 nymphs; cottontail rabbits 56, 349 larvae, 52 nymphs; house cats 22, 189 larvae, 8 nymphs; fox squirrels 24, 6 larvae, 8 nymphs; woodchucks 11, 0 larvae, 1 nymph; opossums 2, 3 larvae, 0 nymphs. Many of these animals were examined when the ticks were either scarce or inactive.

Some information was obtained on the attachment of the larval and nymphal stages of the American dog tick on mice. The percentage infesting the various parts of the body are as follows: larvae: ears, 71.07; cheeks, 10.08; other parts of the head, 8.49; back and shoulders, 5.74; neck, 3.60; legs, .73; sides and belly, .27; nymphs: back and shoulders, 36.80; neck, 22.67; head (except ears and cheeks), 19.70; cheeks, 13.38; ears, 6.69; sides, .74. In general, larvae show a preference for the head, especially the ears, whereas nymphs tend to congregate about the neck, shoulders and forepart of the body.

More than 4,394 animals were examined during the course of the investigation. Included were 266 birds (many ground-inhabiting species) and a number of horses, cattle, and domestic chickens. Some small mammals not mentioned above were also examined, but with negative results.

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THE DISSIMILATION OF GLUCOSE BY CHAETOMIUM FUNICOLA CKE.

III. Some Phosphorus Relationships of *Chaetomium funicola*¹

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INTRODUCTION

The necessity of phosphorus to the development of fungi has been recognized and repeatedly demonstrated since the early investigations of Pasteur (81, 82, 83, 84) and Raulin (87). Although the role that phosphorus assumes in the activity of fungi is still obscure, except with certain yeasts, the possible close relationship of phosphorus to carbohydrate dissimilation for some fungi might be inferred from the increasing demonstrations of phosphorylation in the higher plants (34, 35, 41, 52, 107), yeasts (18, 50, 55, 65, 66, 70, 86), bacteria (5, 79, 116), and animal tissues (4, 16). With such a possible relationship in mind, experiments were conducted with *Chaetomium funicola* Cke. to determine (a) the extent and duration of phosphorus removal from Czapek-Dox medium by developing cultures of the fungus; (b) the formation of a non-orthophosphate P fraction in the medium; (c) the nature of the acid-soluble phosphorus fractions in the mycelium; (d) the interrelationship of the acid-soluble phosphorus fractions in the mycelium through use of macerated mycelial preparations; (e) the respiratory behavior of such mycelial preparations; and (f) the formation of phosphoglyceric acid, uptake of phosphorus, and the formation of methylglyoxal, pyruvic acid, and acetaldehyde by such preparations.

LITERATURE REVIEW

The literature on the phosphorus relations of fungi other than yeasts has been confined largely to *Aspergillus niger*, a fungus which is very different in behavior from *Chaetomium funicola*. A review of some of the available facts concerning this and other fungi is presented here to show the limited scope of the investigations of this subject. Although orthophosphate salts have been used almost exclusively in the cultivation of fungi and in a study of their phosphorus relationships, Coupin (17) and Dox (20) early determined the equal availability to *Aspergillus niger* of

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organic phosphate and inorganic ortho-, pyro-, and metaphosphate salts, the non-availability of hypophosphate, and the toxicity of phosphite and hypophosphite salts. Schnücke (92) obtained the best yields of *A. niger* mycelium on liquid nutrient media containing 0.075 per cent initial concentration of KH_2PO_4 . Koch and Reed (45), on the other hand, obtained progressively lower yields as the initial concentrations of KH_2PO_4 were lowered from 0.5 to 0.05 per cent. Results similar to the latter were obtained by Steinberg (96) for *A. niger* and by Tamiya (102) for *A. oryzae*. Vorbrodt (112) obtained the same ultimate yield of *A. niger* mycelium with 0.5 per cent and lower concentrations of KH_2PO_4 in the medium, except that at concentrations of 0.01 per cent KH_2PO_4 or lower, the yield was much reduced. *Chaetomium funicola* formed maximum yields of mycelium at initial concentrations of 0.1 and 0.025 per cent KH_2PO_4 (94). A close positive relationship between yields of *Aspergillus niger* mycelium and sugar consumed at different initial concentrations of phosphorus was noted by Braun and Frey (9). Waterman (113) noted a close relationship between the concentrations of phosphorus below the optimum and sugar consumed. Semeniuk (94) found greater utilization of sugar per unit dry weight of *Chaetomium funicola* mycelium at the high initial concentrations of phosphorus in the medium.

In relation to products formed by *Aspergillus niger*, Butkewitsch and Timofeeva (11, 12) obtained greater yields of citric acid (with no noticeable influence on oxalic and gluconic acid production) from cultures developing under growth-limiting concentrations of KH_2PO_4 than from cultures developing under adequate concentrations of phosphate for the maximum development of the fungus. Lvoff and Limberg (64) obtained more intense consumption of sugar with greater production of citric acid and later production of oxalic acid by mycelial mats in the presence of added KH_2PO_4 . Gluconic acid production was depressed slightly by the presence of added phosphate. With cultures of *A. oryzae* initiated from spores in media containing M/20, M/40, M/200, and M/1,000 KH_2PO_4 , Tamiya (102) observed marked lower yields of kojic acid with lower concentrations of KH_2PO_4 but ethanol was found only with M/20 and M/40 KH_2PO_4 . Five-day-old mycelial mats placed on media of M/40, M/1,000, and M/0 KH_2PO_4 produced aerobically kojic acid and no ethanol and anaerobically much ethanol but no kojic acid. The presence of inorganic phosphate in the medium either slightly retarded or had no effect on the formation of these products since an abundance of phosphorus was considered to be already contained within the mycelium. With *Rhizopus* sp., Takahashi and Sakaguchi (101) observed ethanol production by submerged mycelium to be greater under lower than average concentration of phosphorus in the medium. Fumaric acid production by surface mycelial mats was greater with higher concentrations of phosphorus in the medium although the production of succinic, malic, and lactic acids remained unaffected.

The disappearance of phosphorus from the medium under developing cultures of *Aspergillus niger* has been accounted for by the total phosphorus appearing in the mycelium (69, 92). The percentage of phosphorus in the mycelium was found to be greater in the earlier stages of fungus

development than in the later stages (92, 112). Higher percentages of total phosphorus in the mycelium occurred with higher initial concentrations of KH_2PO_4 in the medium. Growth-limiting initial concentrations of KH_2PO_4 in the medium yielded mycelium with a stable, low value of 0.36 per cent total phosphorus as P_2O_5 (112). Maximum accumulation of phosphorus in the mycelium was reached at a period of abundant sporulation of the fungus (67, 69, 92) and subsequently decreased with liberation of phosphorus to the medium (67, 69). A reduction of 30 per cent of the phosphorus in *A. niger* occurred in one week of autolysis (69), and a 71 per cent decrease occurred under prolonged cultivation of the fungus for approximately one year (92). *Oidium lactis* and *Dematium pullulans* showed only a slight decrease.

The nature of the phosphorus accumulated in the mycelia of fungi was early noted by Iwanoff (39), for the Agaricaceae, to be organic phosphorus with only small amounts of inorganic phosphorus being present. Zeller (117) recognized these same two phosphorus groups for the higher fungi. He considered the organic phosphorus to be lecithin phosphorus.

Goupil (30) determined mineral, lecithin, and nuclein phosphorus in *Amylomyces rouxii*. Of the total phosphorus in *Aspergillus niger*, Koch and Reed (45) early determined 68.0 per cent extractive (water-soluble) phosphorus, 29.0 per cent protein (nuclein) phosphorus, and 3.0 per cent lecithin phosphorus. Similar values of 60–80 per cent for acid (aqueous) soluble phosphorus were obtained by Vorbrodt (112) and 60–70 per cent by Michel-Durand (69). Vorbrodt considered lecithin phosphorus to be present only in insignificant amounts, but Michel-Durand noted amounts of 5–17 per cent. The extractive or acid-soluble phosphorus as referred to by these latter two investigators was separated further into inorganic and organic phosphorus fractions. The organic phosphorus constituted 0–25 per cent of this fraction as observed by Vorbrodt and 39–67 per cent as determined by Michel-Durand. With yeasts, Macfarlane (65) observed that 50 per cent of the phosphorus contained in these cells was acid-soluble and consisted of 30 per cent inorganic orthophosphate P, 50 per cent labile phosphorus (pyrophosphate), and 20 per cent organic phosphorus. Lohmann (60, 61) found approximately 50 per cent inorganic orthophosphate P, 15–20 per cent pyrophosphate P and the remaining organic phosphorus.

The conditions surrounding the development of *A. niger* have been found to influence the amounts and relative proportions of the different phosphorus constituents in the mycelium. The quantities of acid-soluble and protein phosphorus in the mycelium reached a maximum at a time corresponding approximately to the maximum development (weight) of the mycelium (67, 69, 112). The maximum quantity was lower, however, as the initial concentration of phosphorus in the medium was lower (112). In terms of percentage of dry weight of mycelium, extractive phosphorus and protein phosphorus constituted fairly constant percentages under conditions of 0.5–0.05 per cent initial concentration of KH_2PO_4 in the medium, as observed by Koch and Reed (45). Lower initial concentrations of 0.01–0.005 per cent KH_2PO_4 in the medium resulted in lower per-

centages. Vorbrodt's data, on the other hand, showed progressively lower percentages of these same phosphorus fractions as the initial concentration of KH_2PO_4 was lowered from 0.5 to 0.01 per cent. The protein and acid-soluble phosphorus fractions in this latter work together constituted the total phosphorus in the mycelium, which means, as already pointed out, that the percentage of phosphorus in the mycelium decreased under these conditions.

The ratio of acid-soluble phosphorus to protein phosphorus was lower in the early period of fungus development (112), but such a relationship is not evident in Michel-Durand's data. Also, the influence of initial concentration of phosphorus in the medium on this ratio is not clear since Vorbrodt's data show slightly greater values at very low initial concentrations of 0.025 and 0.01 per cent KH_2PO_4 in the medium while Koch and Reed's data show the reverse.

Lipid phosphorus was found in only trace amounts by Koch and Reed (45) and Vorbrodt (112), but Michel-Durand found lipid phosphorus in the greatest amount at the time of complete sporulation of the mycelium when it represented 17 per cent of the total phosphorus present. A constant low value of 7 per cent was reached after a short period of autolysis.

The constituents of the acid-soluble phosphorus fraction in *A. niger* have been found to undergo considerable fluctuations in relative amount, although the fraction as a whole was found constantly present as 60–80 per cent of the total phosphorus. Since Vorbrodt and Michel-Durand considered only two components, inorganic and organic phosphorus, an increase in one meant a corresponding decrease in the other. Thus, considering only the organic phosphorus fraction, higher proportions of this fraction were obtained in the early stages in the development of *A. niger* up to the time of complete sporulation (69), followed by a decline on autolysis to a lower level. With lower initial concentrations of phosphorus in the medium, lower proportions of organic phosphorus were obtained in the mycelium which in one instance decreased to zero.

MATERIALS AND METHODS

The same culture of *Chaetomium funicola* was used as in the previous studies (93, 94). Other fungi used were: *Fusarium lini*, obtained as culture 1W from Dr. J. J. Christensen, University of Minnesota; *F. oxysporum* var. *cubense*, isolated by Mr. Clifford Meredith from infected banana trees in the West Indies; *F. bulbigenum* var. *niveum*, isolated from wilt-damaged watermelons at Muscatine, Iowa; and *Aspergillus niger*, isolated from apples stored at high temperatures at Ames, Iowa.

The same cultures already reported in experiments 2 and 3 of part 2 of the present series (94) served in the studies of phosphorus removal by developing cultures of *Chaetomium funicola*. Details for the preparations of these cultures were given therein.

The mycelium used in the remaining experiments was initiated from spores at 25–30°C. on Czapek-Dox medium in petri dishes (9 cm. diameter) with approximately 20 cc. medium per dish, maintained in the dark

usually for 6 days.³ The mycelial mats were removed from the desired number of cultures, bulked on a cheesecloth, washed usually three to five times by immersion in as many changes of sterilized distilled water and compressed each time by hand to remove as much of the adhering liquid as possible. Further treatment of the mycelium as to period of storage at 33°F. varied with the different experiments as noted. Immediately before use the mycelium was macerated by grinding in a porcelain mortar with pestle with the aid of silica sand and water to obtain a soupy suspension which could be either (1) poured into a graduated cylinder, (2) easily drawn up into a wide bore pipette, or (3) drawn up into a hypodermic needle. No attempt was made to standardize the relative amounts of mycelium and water in making this macerated mycelial preparation except to add enough water to obtain a fairly thick but workable suspension. Therefore, the results between experiments were not directly comparable although within experiments they were. Steam sterilized utensils were used in all cases to reduce the sources of contamination to a minimum.

The analysis for acid-soluble phosphorus fractions in the mycelium was made on freshly harvested mycelium which was allowed to drain for approximately 10 minutes after bulking, squeezed by hand of adhering liquid medium, then taken to a cold room maintained at 33°F. and there washed three times in as many volumes of cold (33°F.) distilled water. The hand-squeezed mycelium was then placed in a porcelain mortar, flooded with an equal weight of 10 per cent trichloroacetic acid solution, quartz sand added and ground by hand with a pestle to obtain a "soupy" suspension. This suspension was then made to volume with cold 5 per cent trichloroacetic acid solution and allowed to remain in the cold for 4 hours. The liquid portion was then separated from the macerated mycelium by centrifuging and by several passages through filter paper. Phosphorus analyses were made immediately on this filtrate according to the methods indicated below.

Experiments on the interrelationship of the various acid-soluble phosphorus fractions within mycelial preparations were conducted at 30°C. in the dark. Measured volumes of macerated mycelial preparations were transferred to each of a number of previously arranged erlenmeyer flasks containing known volumes of test solutions. Volumes of 15 cc. of the suspension from each experimental flask were removed immediately and at spaced intervals of time thereafter and introduced into 5 cc. of 20 per cent trichloroacetic acid solution. These samples were then placed in a 33°F. room where they were allowed to remain about two days⁴ after all the samples had been taken from any one experiment (usually 24–48 hours' duration). The samples were then treated as a unit, being centri-

³ Period for formation of a surface mycelial mat with sporulation beginning only with *Aspergillus niger*.

⁴ Preliminary trials revealed constant inorganic orthophosphate P values with periods of extraction at 33°F. varying from several minutes to 5 days, while organic phosphorus values showed an increase reaching a maximum after 1 to 2 days' extraction. This latter behavior was obtained only with mycelial preparations which previously had not undergone an autolytic period in a water-toluene mixture (43).

fuged and filtered through filter-paper at the same time, and phosphorus determinations made on the filtrates.

Inorganic orthophosphate P was determined by King's colorimetric method (44) with a Dubosq colorimeter and electric light reflected from a white sheet of smooth paper. Total phosphorus was determined by conversion to inorganic orthophosphate by careful slow ashing in concentrated sulfuric acid over a low flame. Small additions of 30 per cent H_2O_2 were made occasionally to complete the oxidations. Pyrophosphate formed in this operation was converted into the ortho form by hydrolysis of the ashed sample in N HCl for 7 minutes in a boiling water bath after neutralization. Lohmann's method (59, 60, 61, 62) of acid hydrolysis was followed for the phosphorus soluble in the trichloroacetic acid extract, and the phosphorus fractions determined according to the formula used by Macfarlane (65): Labile phosphorus = $\Delta(7' - 0') - \Delta(30' - 7')$; organic phosphorus = total phosphorus - (orthophosphate P at zero minutes hydrolysis + labile phosphorus).

Phosphagen phosphorus determination in the mycelium was made on the cold trichloroacetic acid extract immediately following centrifugation. Eggleton and Eggleton's colorimetric and precipitation methods (22) were followed and the necessary precautions taken for temperature control as pointed out by Fiske and Subbarow (25).

The respiratory activity of macerated mycelial preparations was determined in a Warburg-Barcroft respirometer⁵ shaken at 110 oscillations per minute in a waterbath maintained at $30.4^\circ \pm 0.01^\circ\text{C}$. Atmospheric air only was used in the studies. Small pieces of folded filter-paper were placed in the central cup of concentrated KOH solution in each chamber to facilitate absorption of CO_2 . Observations for the position of the manometer fluid were made once within the first hour and usually every 2 hours thereafter. Aeration of the experimental mixtures in each chamber was provided only at the time of making an observation and readjusting the position of the manometer fluid by a 3-minute opening of the stopcock connecting each chamber to its manometer. No attempt was made to determine the weight of the mycelium introduced into each chamber except that the amount introduced was the same in any one experiment. Each chamber received 0.5 cc. of macerated mycelial preparation to which solutions of various substances were added to make a total volume of 1.5 cc.

Experiments on phosphoglyceric acid formation⁶ and its detection as the barium salt were conducted according to the method of Neuberg and Kobel (72, 73) as modified by Vercellone and Neuberg (111) and by Stone (99). The latter worker observed that holding the flasks containing the experimental mixtures for several days at 5°C . increased the yield of phosphoglyceric acid. The mycelial particles were removed by centrifugation.

⁵ Grateful acknowledgment is here made to Dr. C. H. Werkman of the Bacteriology Department for permission to use this apparatus in his laboratory.

⁶ Appreciation is here expressed to Dr. R. W. Stone, then of the Bacteriology Department, for his assistance in this phase of the work.

gation at the termination of the experiment and the centrifugate analyzed for the presence of the acid.

Analysis for phosphorus uptake by macerated mycelial preparations was made by determining the concentration of inorganic orthophosphate P by the Kuttner and Lichtenstein method (53) in the liquid portion of the experimental mixtures. Samples of the experimental mixtures were removed at intervals, filtered to remove the mycelial particles, and inorganic orthophosphate P determined immediately in their filtrates.

Tests for methylglyoxal, pyruvic acid and acetaldehyde formation by macerated mycelial preparations were carried out following the methods of Simon and Neuberg (95). Mycelial preparations were allowed to remain in suspension of prepared solutions for 48 hours at 28.5°C. at which time the mycelial fragments were centrifuged out and 20 per cent trichloroacetic acid solution added to the centrifugate to make a final concentration of 5 per cent of the acid. Isolations of methylglyoxal, pyruvic acid and acetaldehyde were made as derivatives of 2:4-dinitrophenylhydrazine hydrochloride added in a 2N HCl solution (0.5 gm. of the compound in 60 cc. of hot 2N HCl solution) in proportions of 10 cc. of the compound solution to 50 cc. of the centrifugate. Usually 2-3 hours were allowed for the formation of a precipitate. The precipitate was separated from the mother liquid and fractionated with solvents according to the method of Simon and Neuberg. In one trial methylglyoxal was determined quantitatively by the colorimetric method of Barrenscheen and Dreguss (3), using a standard methylglyoxal solution prepared according to Hoffmann and Neuberg (38) and standardized according to Kühn and Heckscher (51) and Fischler and Boettner (24).

EXPERIMENTAL RESULTS

1. Removal of phosphorus from Czapek-Dox medium by developing cultures of *Chaetomium funicola*.

The data for inorganic and total phosphorus in the medium and for total phosphorus in the mycelium under conditions of different initial concentrations of KH_2PO_4 and pH are presented in tables 1 and 2, respectively. With normal (0.1 per cent) or greater concentrations of KH_2PO_4 , *C. funicola* continued to remove phosphorus from the medium over the entire period of 23 days. Varying the initial pH of the medium did not influence this behavior except under initially alkaline and very acid conditions, where the amounts of mycelium formed and phosphorus removed were retarded. Greater removal of phosphorus from the medium occurred with higher concentrations of phosphorus, yielding correspondingly higher total and percentage phosphorus contents in the mycelium. With 0.025 per cent initial concentration of KH_2PO_4 the inorganic orthophosphorus supply in the medium was depleted after approximately 13 days, but the plants still continued to produce mycelium. After 23 days as much mycelium was produced as in cultures on 0.1 per cent initial concentration of KH_2PO_4 which still retained much inorganic phosphorus in the medium. With no phosphate added to the medium, development of *C.*

TABLE 1
ANALYSIS OF *Chaetomium funicola* CULTURES DEVELOPED ON CZAPEK-DOX MEDIUM
WITH DIFFERENT INITIAL CONCENTRATIONS OF KH_2PO_4

Days of Fungus Development	Initial Percentage Concentration of KH_2PO_4 in the Medium						
	0	0.025	0.1	0.3	0.6	1.5	3.0
Mycelium Formed, Milligrams							
6.....	0.6	60.0	82.3	79.4	87.6	64.5	56.2
13.....	35.7	287.6	267.8	260.8	233.2	185.4	209.2
22.....	40.8	400.8	418.0	339.3	355.3	354.4	333.4
Inorganic Orthophosphate P in the Medium, Milligrams							
0.....	0.0	1.8	7.0	21.1	42.6	104.5	211.3
6.....	0.0	1.4	5.9	19.8	40.9	106.6	210.6
13.....	0.0	Trace	4.1	15.3	36.7	99.7	196.1
22.....	Trace	0.2	3.9	13.8	33.6	97.8	187.2
Total Phosphorus in the Medium, Milligrams							
0.....	Trace	8.0	21.9	43.0	106.6	210.0
6.....	1.0	1.8	6.5	19.6	41.2	107.0	208.4
13.....	0.7	0.8	5.3	18.0	38.7	103.9	205.5
22.....	0.7	1.0	5.7	16.4	33.7	91.0	181.4
Total Phosphorus in the Mycelium, Percentage							
13.....	0.3	0.7	0.8	1.0	1.6	1.8	3.9
22.....	0.8	1.4	1.8	1.8	2.1	3.0

funicola was limited, suggesting the utilization of the phosphorus contained in the spores originally sown in the medium. Rennerfelt (88) found a higher percentage of phosphorus in the spores of *Aspergillus niger* than in the mycelium. Phosphorus constituted 5.77 per cent of the ash in spores and only 1.84 per cent of the ash in the mycelium.

Although the values for total phosphorus in the mycelium are not in complete agreement with the decrease in total phosphorus in the medium, the discrepancies obtained are similar to those already recorded for *Aspergillus niger* (69). In general, however, the phosphorus analyses in the mycelium support the observations already made of the disappearance of phosphorus from the medium.

2. Formation of a non-orthophosphate P fraction in Czapek-Dox medium.

The presence of a non-orthophosphate P fraction in the medium, as determined by difference between the values for total and inorganic orthophosphate, is suggested more convincingly by the data in table 2 than by those in table 1. Because of the necessity of varied dilutions of the media according to the concentration of phosphorus present, certain undetermined variable errors were introduced into the phosphorus values shown in table 1. In this experiment, in addition, no step was taken to reduce to orthophosphate any pyrophosphate formed during ashing of the sample in the determinations of total phosphorus. Accordingly, some of the total phosphorus values are lower than the corresponding inorganic phosphorus values. An explanation other than errors in dilutions cannot

TABLE 2

ANALYSIS OF *Chaetomium funicola* CULTURES DEVELOPED ON CZAPEK-DOX MEDIUM WITH DIFFERENT INITIAL pH VALUES AND WITH 0.1 PER CENT INITIAL CONCENTRATION OF KH_2PO_4

Days of Fungus Development	Initial pH of Culture Medium							
	2.90	4.03	4.98	6.05	7.20	7.78	8.15	8.68
Mycelium Formed, Milligrams								
10.....	27.5	211.8	195.8	183.1	173.8	99.8	45.9	16.3
23.....	33.0	410.7	270.6	355.9	521.5	208.6	383.6	180.2
Inorganic Orthophosphate P in the Medium, Milligrams								
0.....	7.5	7.3	8.1	8.1	7.8	7.5	8.1	7.0
10.....	6.8	5.2	4.7	5.7	6.0	6.2	6.5	6.8
23.....	5.5	2.3	2.3	2.1	2.9	4.2	3.6	4.2
Total Phosphorus in the Medium, Milligrams								
0.....	8.1	7.8	8.1	8.1	7.8	7.5	7.5	6.8
10.....	7.8	6.5	6.5	6.8	7.5	7.8	9.4	8.3
23.....	6.0	3.9	3.9	3.4	3.6	4.9	5.2	4.7
Total Phosphorus in the Mycelium, Percentage								
10.....		1.4	1.3	1.0	0.9	0.8	0.8	1.1
23.....	0.9	0.7	1.0	0.9	0.6	0.7	0.6	0.9

be offered for the reverse situation where inorganic orthophosphate P is lower than total phosphorus as obtained in the analysis at zero time. Non-orthophosphate P in the medium was indicated more consistently in cultures with low initial concentration of phosphorus in the medium. Furthermore, the liberation of non-orthophosphate P from the mycelium is suggested by the cultures with no additions of KH_2PO_4 and by the older cultures initially supplied with 0.025 per cent KH_2PO_4 .

In the experiment represented by the data in table 2, a consistently significant non-orthophosphate P fraction was detected in the medium after 10 and 23 days of fungus development in contrast to the small positive and negative values attributable to errors in method obtained at zero days' development. Thus, while the net non-orthophosphate P fraction at zero time analysis represented 1 per cent of the total phosphorus present in the medium, at 10 and 23 days this fraction represented 20 and 21 per cent, respectively. The presence of a non-orthophosphate P fraction in the medium was further supported by the results of analysis by Lohmann's hydrolysis method of the phosphorus in the filtrate from 14-day-old cultures grown in petri dishes. This filtrate tested per cc. 115.0 gamma total phosphorus of which 95.6 gamma were inorganic orthophosphate P, 12.7 gamma labile phosphorus, and 6.7 gamma organic phosphorus. The organic phosphorus was hydrolyzed to the extent of 40 per cent in 3 hours. This observation was further confirmed by methods designed to precipitate and to characterize the organic phosphorus fraction.

In the first trial, adapting the method of Tanko (107), 300 cc. of Czapek-Dox solution filtrate obtained from 6-day-old petri dish cultures and made alkaline to phenolphthalein was treated in slight excess with

basic lead acetate. The precipitate was centrifuged off, washed several times with cold water, resuspended in water, and decomposed in an ice bath by additions of a dilute solution of H_2SO_4 . The precipitate formed was centrifuged off and discarded. Barium hydroxide was then added to the centrifugate until no further precipitation occurred, and was followed by 2.5 volumes of 95 per cent ethanol. The precipitate was centrifuged off, washed successively with 70 and 95 per cent ethanol and dried at 50°C . Although most of this precipitate was made up of BaSO_4 , a portion soluble in 0.1 N H_2SO_4 solution yielded a phosphorus fraction containing approximately 50 per cent inorganic orthophosphate P with the remainder being undetermined. Hexosediphosphate was not detected as a constituent of this undetermined phosphorus fraction.

In a second trial the same amount of solution used in the first was obtained from 18-day-old cultures. Of the total phosphorus present in solution, 24.7 per cent was in the non-orthophosphate form. The solution was made alkaline to litmus and treated in excess with basic lead acetate. The precipitate formed was washed three times by suspension in water and centrifuging, then suspended in water, decomposed with H_2S , and filtered. The filtrate was made alkaline to litmus, and the inorganic phosphorus precipitated with magnesium acetate and filtered out. The filtrate (80 cc.) was made acid by addition of 2 cc. glacial acetic acid, and 5 cc. of 20 per cent barium acetate solution were added. The precipitate was allowed to form over night at 33°F ., centrifuged off, and washed in succession with 2 per cent glacial acetic acid, water, ethanol, and acetone. A light powder weighing 80.3 mgms. was obtained which gave a positive test for phosphorus and nitrogen, liberated H_2S on treatment with dilute H_2SO_4 , and burned quickly to a metallic residue. Extraction of the powder with 0.1 N H_2SO_4 solution revealed 0.48 per cent total phosphorus in the powder, of which 44.0 per cent was inorganic orthophosphate P, 6.7 per cent labile phosphorus, and 49.3 per cent organic phosphorus. The organic phosphorus was hydrolyzed to the extent of 30 per cent in 30 minutes with no additional hydrolysis on further heating to 60 minutes. To the filtrate (87 cc.) yielding the above light powder were added 11 cc. of 95 per cent ethanol to make the concentration of the latter 10 per cent. No precipitate was formed. Ninety cc. of 95 per cent ethanol were then added and an abundant, flocculent precipitate was formed. The precipitation was allowed to proceed over night at 33°F . after which the precipitate was centrifuged off, washed in 50 and 95 per cent ethanol, and dried in air. A faintly yellow powder was obtained weighing 302.9 mgms. which gave a positive test for nitrogen and phosphorus and possessed an iodine-reducing power equivalent to 1.5 per cent glucose content. Barium made up 38.3 per cent of the weight of this powder while total phosphorus as extracted for the powder with 0.1 N H_2SO_4 made up 5.2 per cent. Organic phosphorus constituted 35.0 per cent and labile phosphorus 2.6 per cent of the total phosphorus, the remaining 62.3 per cent being inorganic orthophosphate P. Ninety per cent of the organic phosphorus in this fraction was resistant to 3 hours' hydrolysis by N HCl at 100°C .

3. Nature of the acid-soluble phosphorus fractions in the mycelium.

An analysis of the acid-soluble phosphorus in the mycelium of *C. funicola* and four other fungi is shown in table 3. The combined mycelia from 20 6-day-old petri-dish cultures for each fungus used, were adjusted to 100 cc. volume with a trichloroacetic acid solution. The total acid-soluble phosphorus was definitely much higher with the three *Fusarium* spp. than with the other two fungi. The order of the fungi for total acid-soluble phosphorus in the mycelium from the least to the greatest was *Aspergillus niger*, *Chaetomium funicola*, *Fusarium lini*, *F. niveum*, and *F. cubense*. The amount of inorganic orthophosphate P varied with the different fungi according to the total acid-soluble phosphorus present, rep-

TABLE 3
ANALYSIS FOR PHOSPHORUS FRACTIONS IN THE TRICHLOROACETIC ACID EXTRACTS
OBTAINED FROM THE MYCELIA OF FIVE FUNGI

Time of Hydrolysis in N HCl at 100°C.	Orthophosphate P (Gamma) per 5 cc. of Acid Extract				
	<i>Aspergillus niger</i>	<i>Chaetomium funicola</i>	<i>Fusarium lini</i>	<i>Fusarium cubense</i>	<i>Fusarium niveum</i>
0...	6.0	20.2	46.1	37.3	61.4
7...	12.8	69.5	113.0	192.2	219.5
30...	14.0	69.5	111.7	208.2	227.5
180...	15.4	71.5	118.5	208.2	236.0
Total phosphorus.....	30.0	99.0	171.2	565.0	305.0
Inorganic orthophosphate P percentage.....	20.0	20.4	26.9	6.6	20.1
Labile phosphorus percentage.....	18.7	49.8	39.1	24.6	49.2
Organic phosphorus percentage.....	61.3	29.8	34.0	68.8	30.7
Percentage organic phosphorus hydrolyzed in 3 hrs.....	12.7	6.8	9.5	8.2	26.2

representing a fraction of approximately 20 per cent of the total. *Fusarium cubense*, however, contained inorganic orthophosphate P equal to only 6.6 per cent of the total acid-soluble phosphorus present. Labile phosphorus was 0.9, 2.4, 1.4, 3.7, and 2.4 times greater than the inorganic orthophosphate P for each of the fungi *Aspergillus niger*, *Chaetomium funicola*, *Fusarium lini*, *F. cubense*, and *F. niveum*, respectively. Together, the inorganic and labile phosphorus constituted 38.7, 70.2, 65.9, 31.2, and 69.4 per cent of the total phosphorus for each of the fungi, the remainder being organic phosphorus. The organic phosphorus for the most part was resistant to 3 hours' acid hydrolysis since for each of the above fungi the extent of hydrolysis attained was 12.7, 6.8, 9.5, 8.2, and 26.2 per cent. Phosphagen phosphorus was not detected.

The influence of age of *Chaetomium funicola* mycelium on the composition of the acid-soluble phosphorus fractions is shown by the data in table 4 and by figure 1. Washed and hand pressed mycelia from 40 petri dish cultures at two ages of development were made to 200 cc. with

TABLE 4

ANALYSIS FOR PHOSPHORUS FRACTIONS IN THE TRICHLORACETIC ACID EXTRACTS OBTAINED FROM MYCELIA OF 8- AND 18-DAY-OLD CULTURES OF *Chaetomium funicola*

Time of Hydrolysis in N HCl at 100°C.	Orthophosphate P (Gamma) per 2.5 cc. of Acid Extract	
	Age of Cultures	
	8 days	18 days
0.....	57.2	54.3
7.....	111.0	320.0
30.....	152.0	409.0
180.....	164.0	422.5
Total phosphorus.....	207.0	486.5
Inorganic orthophosphate P percentage.....	27.6	11.2
Labile phosphorus, percentage.....	6.2	36.3
Organic phosphorus, percentage.....	66.2	52.5
Percentage organic phosphorus hydrolyzed in 3 hours.....	68.6	75.0

trichloroacetic acid solution. Total acid-soluble phosphorus was greater by 2.3 times in the 18-day-old mycelia than in the 8-day-old samples. Inorganic orthophosphate P was the same at both ages while labile phosphorus and organic phosphorus were greater by 5.9 and 0.8 times, respectively. As percentages of total phosphorus, inorganic orthophosphate P was lower by 60 per cent, labile phosphorus higher by 110 per cent and organic phosphorus lower by 27 per cent in the older mycelia. The percentage values of the total phosphorus for inorganic orthophosphate P, labile phosphorus, and organic phosphorus in the older mycelia were 27.6, 6.2, and 66.2, respectively. The hydrolysis curve suggests that the organic phosphorus fraction was similar in both ages of mycelium. This fraction liberated approximately 70 per cent of its phosphorus in 3 hours' hydrolysis, but 90 per cent of this was liberated during the first 30 minutes of hydrolysis. The possibilities are that, either the liberation of phosphorus from the labile phosphate fraction was incomplete after 7 minutes' hydrolysis, or the organic phosphorus fraction was composed of at least two fractions, one of which possessed a phosphorus labile to acid hydrolysis, the other a phosphorus that was quite stable.

4. Interrelationship of the acid-soluble phosphorus fractions in the mycelium.

The presence of the different acid-soluble phosphorus fractions in the mycelium of fungi suggested an investigation of a possible interrelationship like that recently found with yeasts, bacteria, blood cells, and various animal tissues. For this purpose use was made of macerated mycelial preparations of *C. funicola* and several other fungi prepared from freshly harvested 6-day-old mycelium to which different substances were added. The changes in phosphorus obtained at chosen intervals of time revealed

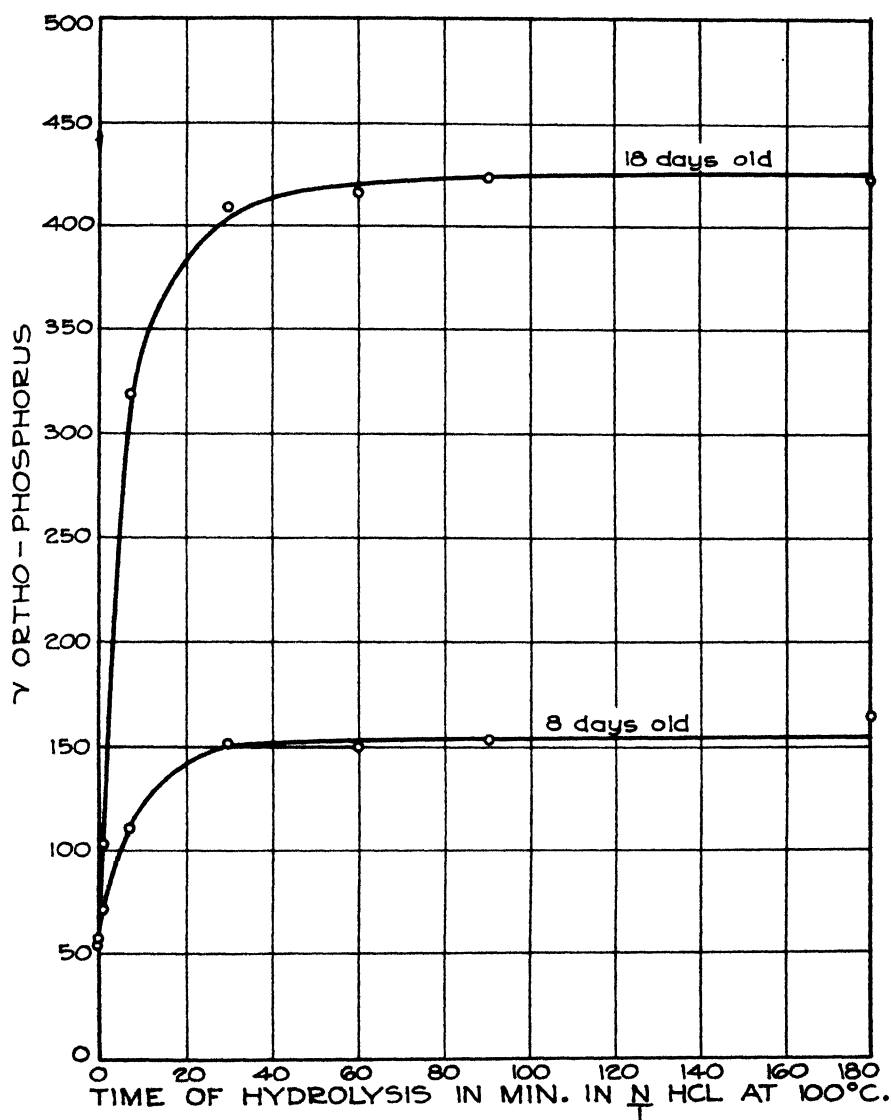


FIG. 1. Hydrolysis curves of the acid-soluble phosphorus fraction obtained from *Chaetomium funicola* mycelia of two different ages.

autolysis to be the predominating if not the exclusive reaction of these preparations under the conditions employed. Figure 2 presents the changes obtained in an initial experiment in inorganic orthophosphate phosphorus and pyrophosphate phosphorus. Inorganic orthophosphate phosphorus increased with time, reaching a maximum in approximately 20 hours in all the experimental treatments. Glucose retarded phosphorus mineralization in the absence of sodium fluoride and toluene but

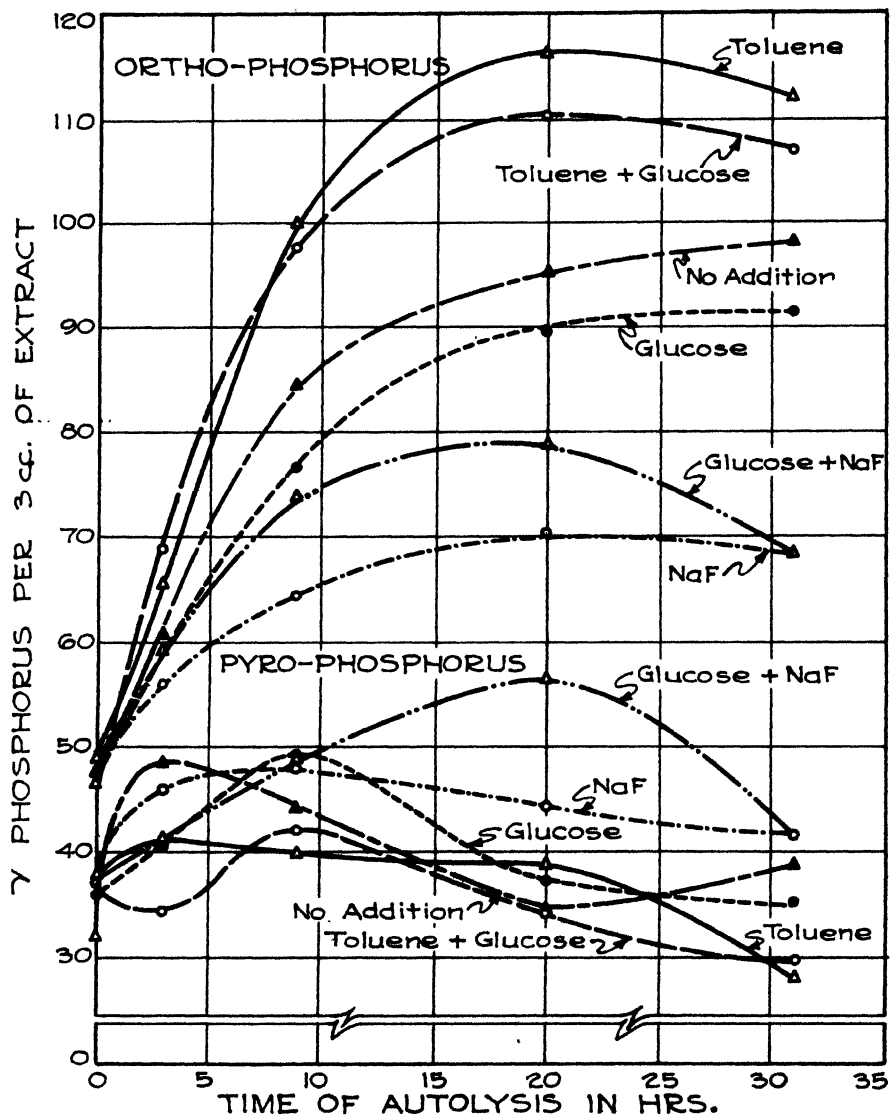


FIG. 2. Changes in two acid-soluble phosphorus fractions during autolysis of *Chaetomium funicola* mycelial preparations.

enhanced it in the presence of sodium fluoride and in the early period with toluene. Sodium fluoride exerted a retarding action, and toluene favored phosphorus mineralization in the absence and presence of added glucose. The pyrophosphate phosphorus fraction ($7'-0'$) increased with time and then decreased, except in the presence of toluene and glucose, where an initial decrease was obtained. The presence of glucose retarded by several hours the attainment of the maximum pyrophosphate values.

The maximal pyrophosphate values attained with no treatment or with toluene treatment were the same in the presence or absence of glucose, but were much greater in the presence of glucose with sodium fluoride.

TABLE 5

PROGRESSIVE CHANGES DURING AUTOLYSIS IN THE ACID-SOLUBLE PHOSPHORUS FRACTIONS OF MYCELIAL PREPARATIONS OF *Chaetomium funicola** UNDER ADDITIONS OF TOLUENE AND NaF

Experimental Mixtures †	Phosphorus Fractions	Gamma Phosphorus per 2.5 cc. Acid Extract			
		Initial Values	Increases After Autolytic Periods (hrs.)		
			2¼	10	18
1. No additions	Total-P.	113.0	10.0	17.5	22.1
	Inorg.-P.	44.1	8.9	28.3	42.3
	Labile-P.	30.9	- 2.9	- 4.0	- 4.9
	Organic-P-1 ‡ . .	16.2	- 2.8	-15.5	-16.2
	Organic-P-2 § . .	21.8	6.8	8.7	0.9
2. Toluene	Total-P.	115.2	13.6	25.1	25.1
	Inorg.-P.	41.2	7.4	50.5	62.9
	Labile-P.	31.6	4.2	-16.3	-14.7
	Organic-P-1 ‡ . .	13.8	- 3.8	- 8.8	-12.3
	Organic-P-2 § . .	28.6	5.8	- 0.3	-10.8
3. NaF	Total-P.	113.6	7.6	10.5	11.4
	Inorg.-P.	45.4	10.6	20.1	31.4
	Labile-P.	28.0	- 3.6	- 6.9	- 3.0
	Organic-P-1 ‡ . .	21.0	- 5.9	- 7.7	-15.2
	Organic-P-2 § . .	19.2	6.5	5.0	- 1.8

* Mycelium obtained from 78 petri dish cultures, grown 6 days, washed twice and macerated to a volume of 240 cc.

† Exp. mixture 1. 80 cc. mycelial preparation 160 cc. water

2. " " " " 160 cc. water, 1 cc. toluene

3. " " " " 160 cc. water, 48 cc. 0.2M NaF

‡ Organic phosphorus hydrolyzed by 3 hours' acid hydrolysis.

§ Organic phosphorus not hydrolyzed by 3 hours' acid hydrolysis.

Pyrophosphate accumulation was greater with sodium fluoride and lower with toluene than when both were present.

A comparison of the changes in the different acid-soluble phosphorus fractions in the presence and absence of toluene and sodium fluoride (Table 5) with and without glucose (Table 6) revealed progressive increases in total acid-soluble phosphorus and inorganic orthophosphate P, general decreases in labile phosphorus and 3-hour acid-hydrolyzable organic phosphorus, and initial increases with subsequent decreases in 3-hour acid-unhydrolyzable organic phosphorus. Maximal increases of 10-27 per cent and 70-200 per cent were obtained for total acid-soluble phosphorus and inorganic orthophosphate P, respectively. In general, toluene enhanced the rate and extent of phosphorus conversions while sodium fluoride exerted a retardation action. Glucose only slightly increased these conversions in the presence of these compounds.

In other experiments additions of inorganic orthophosphate buffer (pH 6.85), alone or in the presence of sodium fluoride or sodium fluoride plus glucose, did not alter appreciably the general behavior of the various

phosphorus fractions. Respiratory poisons, as potassium cyanide and ethyl urethane, phosphorylation-inhibiting phlorhizin, and iodoacetate, likewise exerted no noticeable influence on the changes obtained in the phosphorus fractions. Further, the same general behavior of phosphorus

TABLE 6

PROGRESSIVE CHANGES DURING AUTOLYSIS IN THE ACID-SOLUBLE PHOSPHORUS FRACTIONS OF MYCELIAL PREPARATIONS OF *Chaetomium funicola** UNDER ADDITIONS OF TOLUENE AND NaF WITH AND WITHOUT GLUCOSE

Experimental Mixtures†	Phosphorus Fractions	Gamma Phosphorus per 2.5 cc. Acid Extract					
		Initial Values	Increases After Autolytic Periods (Hrs.)				
			2½	5	8	12	17
1. NaF	Total-P.	110.0	- 1.5	5.0	11.0	16.8	16.5
	Inorg.-P.	34.0	6.4	10.8	16.6	27.5	34.7
	Labile-P.	9.2	- 2.7	- 2.7	0.3	- 5.3	- 7.4
	Organic-P-1‡. . .	23.6	- 5.6	- 8.2	-13.7	-11.9	-14.5
	Organic-P-2§. . .	43.2	0.4	5.1	7.8	6.5	3.7
2. NaF plus glucose	Total-P.	114.5	0.5	7.4	18.7	14.3	23.0
	Inorg.-P.	34.1	8.1	14.3	21.9	38.0	50.1
	Labile-P.	8.2	- 3.4	- 3.6	- 0.3	- 7.7	- 9.2
	Organic-P-1‡. . .	25.1	- 3.2	- 5.6	-11.1	-11.9	-16.1
	Organic-P-2§. . .	47.1	- 1.0	2.3	8.2	- 4.1	- 1.8
3. Toluene	Total-P.	119.0	2.0	16.1	26.0	28.8	31.0
	Inorg.-P.	38.5	18.4	29.8	44.7	63.0	76.7
	Labile-P.	12.9	- 3.6	-11.2	-11.6	- 4.4	- 5.1
	Organic-P-1‡. . .	22.1	- 7.3	10.6	- 1.4	-15.0	-25.1
	Organic-P-2§. . .	45.5	- 0.5	-13.1	- 5.7	-14.8	-15.5
4. Toluene plus glucose	Total-P.	117.2	15.1	18.8	27.8	29.8	32.3
	Inorg.-P.	38.4	19.1	34.5	53.5	72.4	75.1
	Labile-P.	7.7	- 0.7	- 2.9	- 5.8	1.0	1.3
	Organic-P-1‡. . .	23.6	- 8.5	- 1.8	- 9.2	-25.1	-22.7
	Organic-P-2§. . .	47.5	5.2	-11.1	-10.7	-18.5	-21.4

* Mycelium obtained from 80 petri dish cultures, grown 6 days, washed three times and macerated to a volume of 240 cc.

† Exp. mixture 1. 60 cc. mycelial preparation 12 cc. 0.2M NaF, 48 cc. water.

2. " " " " 12 cc. 0.2M NaF, 18 cc. water, 30 cc. 10%

3. " " " " 1 cc. toluene, 59 cc. water. [glucose.

4. " " " " 1 cc. toluene, 29 cc. water, 30 cc. 10% glu-

‡ Organic phosphorus hydrolyzed by 3 hours' acid hydrolysis. [cose

§ Organic phosphorus not hydrolyzed by 3 hours' acid hydrolysis.

as with *C. funicola* was obtained with macerated mycelia of other fungi; namely, *Fusarium niveum*, *F. lini*, and *F. oxysporum* in the presence and absence of toluene or toluene plus sodium fluoride.

5. Respiratory activity of macerated mycelial preparations of *Chaetomium funicola*.

The foregoing mineralization of phosphorus in macerated mycelial preparations of *C. funicola* suggested a determination of the respiratory activity of such preparations as measured in a Warburg-Barcroft respiro-

TABLE 7
RESPIRATORY ACTIVITY OF MACERATED MYCELIAL PREPARATIONS OF *Chaetomium funicola**

Additions Made to Mycelial Preparations	Readings	Respiratory Quotient and cmm. CO ₂ Evolved per Hour During Different Time Intervals (hrs.)																		Total CO ₂ Evolved
		0-1 1/4	1 1/4-2 1/4	2 1/4-3 1/4	3 1/4-4 1/4	4 1/4-5 1/4	5 1/4-6 1/4	6 1/4-7 1/4	7 1/4-8 1/4	8 1/4-9 1/4	9 1/4-10 3/4	10 3/4-11 1/4	11 1/4-12 1/4	12 1/4-14	14-15 1/4	15 1/4-16 3/4	16 3/4-18 1/4	18 1/4-20 1/4	20 1/4-22 1/4	
None.....	CO ₂ R. Q.	20.4 1.26	13.8 .95	25.5 .98	66.9 1.29	104.0 1.17	44.5 .77	24.0 .55	26.5 .58	29.0 .77	31.8 .87	30.0 .68	33.4 .92	30.0 .68	26.3 .92	31.8 .87	30.0 .68	33.4 .92	26.3 .92	1,019.9
Glucose.....	CO ₂ R. Q.	20.0 1.11	16.5 .94	34.1 1.02	86.4 1.13	111.0 1.37	135.0 1.25	110.0 1.23	119.3 1.25	113.0 1.24	144.7 1.85	97.5 1.23	80.0 1.01	97.5 1.23	80.0 1.01	144.7 1.85	97.5 1.23	80.0 1.01	27.9 4.88	1,859.1
NaF M/300.....	CO ₂ R. Q.	21.3 1.13	15.6 .94	28.5 1.00	83.2 1.24	94.0 1.09	77.9 .80	45.4 .83	39.2 .87	32.7 .84	33.6 .90	26.0 .87	34.1 .93	26.0 .87	34.1 .93	33.6 .90	26.0 .87	34.1 .93	25.8 .92	983.0
Glucose + NaF M/300.....	CO ₂ R. Q.	14.8 1.32	13.5 .93	34.7 1.09	82.0 1.03	74.5 1.05	68.7 1.00	24.9 .74	21.8 .89	17.3 .88	14.4 .94	12.9 1.01	12.9 1.01	12.9 1.01	12.8 1.33	14.4 .94	12.9 1.01	12.9 1.01	12.8 1.33	735.9
Iodoacetate M/300.....	CO ₂ R. Q.	23.5 1.72	14.8 1.04	23.4 .94	37.3 1.07	42.3 1.11	52.7 1.11	50.1 1.08	59.6 1.11	52.6 1.03	50.6 1.03	46.8 .93	46.8 .97	46.8 .97	30.7 .92	50.6 1.03	46.8 .93	46.8 .97	30.7 .92	927.5
Glucose + Iodoacetate M/300.....	CO ₂ R. Q.	23.5 2.18	12.8 1.11	26.7 1.02	32.3 2.07	46.8 1.09	56.8 1.10	61.8 1.01	40.9 .74	35.4 .68	34.0 .71	13.8 .52	14.2 .82	14.2 .82	50.2 .74	34.0 .71	13.8 .52	14.2 .82	50.2 .74	787.9
Oxalate M/300.....	CO ₂ R. Q.	10.7 1.25	13.4 .94	35.1 1.40	149.0 1.01	107.2 1.14	76.3 1.11	47.5 1.15	42.4 1.20	43.7 1.19	45.8 1.32	41.7 1.20	44.7 1.21	44.7 1.21	32.0 .86	43.7 1.19	41.7 1.20	44.7 1.21	32.0 .86	1,239.2
Glucose + Oxalate M/300.....	CO ₂ R. Q.	14.5 .97	15.8 .88	52.1 1.14	107.8 1.01	132.8 1.14	162.2 1.11	128.5 1.10	124.5 1.09	118.5 1.09	108.0 1.14	107.5 1.12	107.5 1.12	107.5 1.12	79.1 1.09	108.0 1.14	107.5 1.12	107.5 1.12	79.1 1.09	2,188.9

* Fungus grown 6 days; mycelium freed of reducing sugars by washing in running tap water over a 12-hour period. Mycelial preparation made from 10 gm. moist material + 15 cc. water. Additions to mycelial preparation in each chamber: 0.5 cc. 15% glucose solution; 0.5 cc. 0.01M solutions of NaF, iodoacetate or oxalate, adjusted to pH 6.7, water to make 1.5 cc. total volume.

meter. Use was made of poisons of known action (7, 15, 19, 27) to learn something of the nature of the respiratory activity.

The respiratory activity data for mycelial preparations to which were

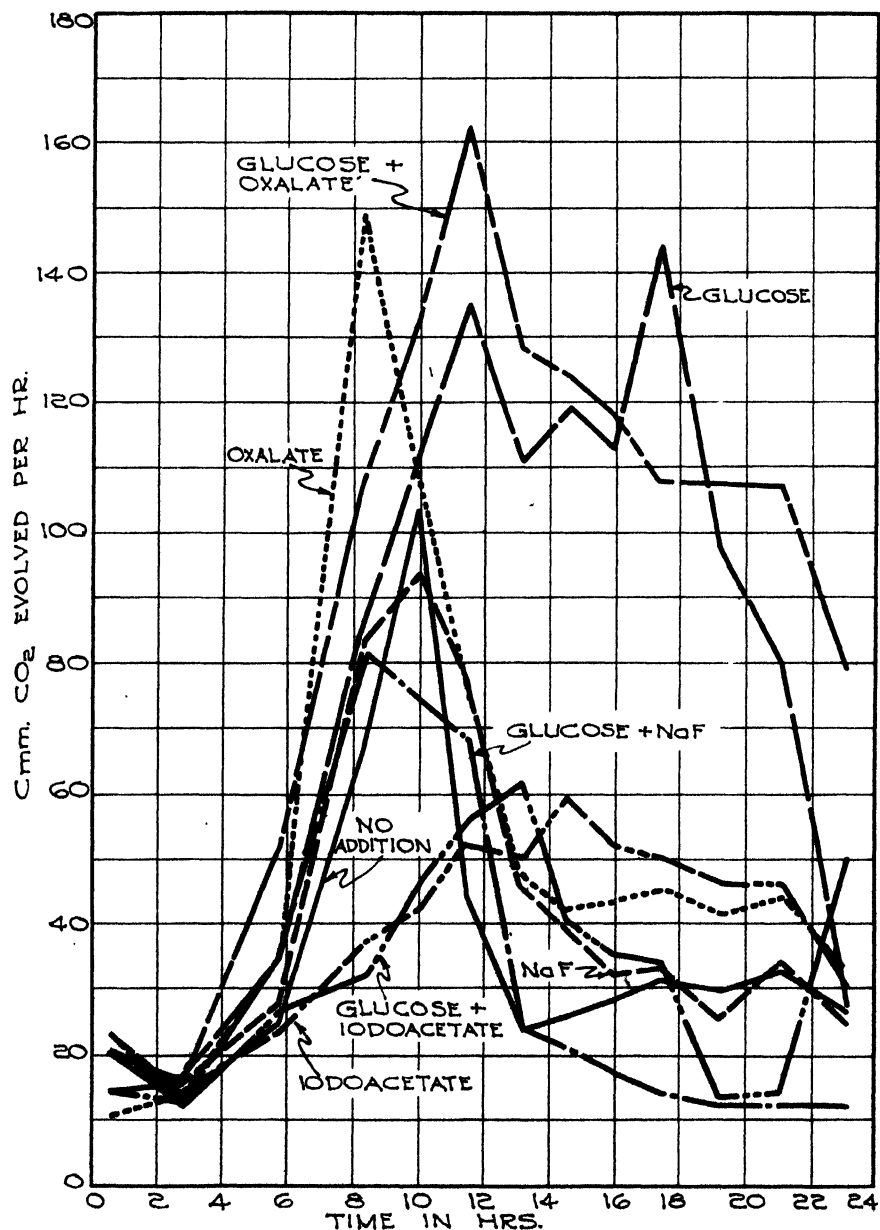


FIG. 3. Respiratory activity of macerated mycelial preparations of *Chaetomium funicola*.

added glucose, NaF, iodoacetate, and oxalate in several combinations are presented in table 7 and figure 3. These data reveal: (1) an initial induction period; (2) endogenous and exogenous respiratory activity (97, 98); (3) inhibition of exogenous activity by NaF and iodoacetate, with no effect on endogenous activity by NaF but with some inhibition by iodoacetate; and (4) slight stimulatory effect of oxalate on endogenous and exogenous activity.

The depressive effect of iodoacetate on endogenous activity and the complete suppression of exogenous activity was different from that reported for "*Fusarium* sp. H" (29) but in line with the concept that this compound interferes with the oxido-reduction processes (specifically, with triosephosphate dehydrogenase); more with fermentation than with respiration (110). Sodium fluoride is known to be a general inhibitor of esterase (including phosphatase) enzyme activity (21, 56, 57, 68) and particularly of the enolase enzyme converting 2-phosphoglyceric acid to 2-phosphopyruvic acid (114). The stimulatory effect of oxalate was in line with the suggestion (54) that it retards co-enzyme destruction, although earlier work showed an inhibitory effect on glycolysis, specifically the conversion of phosphopyruvate to pyruvate and phosphate (63). Tamiya (102) reported a similar stimulatory effect for *Aspergillus oryzae*.

Further evidence for the distinction between endogenous and exogenous activities is supported by the effect of decreasing strengths of iodoacetate; namely M/30, M/100 and M/300 (Table 8). Complete inhi-

TABLE 8
IODOACETATE INHIBITION OF RESPIRATORY ACTIVITY BY MYCELIAL
PREPARATIONS OF *Chaetomium funicola**

Iodoacetate Concentration	Total cmm. CO ₂ Evolved†	
	With Glucose	Without Glucose
0.....	559.9	128.7
M/30.....	4.6	0.0
M/100.....	28.7	10.3
M/300.....	60.2	103.6

* Fungus grown 13 days; mycelial preparation made from 10 gm. moist mycelium + 15 cc water. Additions to mycelial preparation in each chamber: 0.5 cc. 15% glucose solution 0.5 cc. iodoacetate solutions adjusted to pH 6.7 and water to make 1.5 cc. total volume
† Over a 6-hour period.

bition of exogenous activity was obtained with all strengths of iodoacetate, but inhibition of endogenous activity was milder with weaker strengths of the inhibitor.

Cyanide in strengths of M/1,000 and M/500 (Table 9) almost completely suppressed endogenous and exogenous activities (O₂ consumption to a slightly greater extent than CO₂ evolution) thereby suggesting that both processes are oxidative in nature. This effect on exogenous activity is different from that recorded for "*Fusarium* sp. H" (29) and yeast (98) where CO₂ evolution remained unaffected or affected to only a slight

TABLE 9
CYANIDE AND URETHANE INHIBITION OF RESPIRATORY ACTIVITY BY MYCELIAL
PREPARATIONS OF *Chaetomium funicola**

Poison Added	Concentration	Total cmm. CO ₂ Evolved and O ₂ Consumed †			
		With Glucose		Without Glucose	
		CO ₂	O ₂	CO ₂	O ₂
None.....		568.2	555.4	130.0	117.0
KCN.....	0.001M	98.4	77.8	26.2	19.6
KCN.....	0.002M	58.8	41.3	14.0	14.0
Urethane.....	0.57M	31.9	29.6	45.8	

* Fungus grown 7 days; mycelium washed 3 times, let stand overnight at 33°F. then washed twice. Mycelial preparation made from 15 gm. moist mycelium + 20 cc. water. Additions to mycelial preparation in each chamber: 0.5 cc. 15% glucose solution, 0.5 cc. solutions of KCN and urethane and water to make 1.5 cc. total volume.

† Over a 12-hour period.

TABLE 10
INFLUENCE OF INORGANIC PHOSPHATE AND TOLUENE ON THE RESPIRATORY ACTIVITY BY
MYCELIAL PREPARATIONS OF *Chaetomium funicola**

Additions	Total cmm. CO ₂ Evolved †	
	With Glucose	Without Glucose
None.....	1169.5	802.0
Phosphate.....	1066.3	1010.0
Toluene.....	18.2	52.5
Phosphate + toluene.....	35.9	30.9

* Fungus grown 6 days; mycelium washed free of reducing sugars and stored overnight at 33°F. Mycelial preparation made from 10 gm. moist mycelium + 15 cc. water. To mycelial preparation in each chamber added 0.5 cc. 15% glucose solution, 0.5 cc. phosphate buffer (2:1 of 2/3M K₂HPO₄ and 2/3M KH₂PO₄, respectively) pH 6.85 and water to make 1.5 cc. total volume. Six drops toluene added where indicated.

† Over a 14-hour period.

extent. A small residual exogenous activity over endogenous activity in the presence of cyanide might be indicative of a weakly anaerobic fermentative process such as has been found for a number of different fungi (109). Urethane 0.57M (Table 9) almost completely inhibited endogenous and exogenous activities in conformity with its behavior as a general poison of the dehydrogenase enzyme systems (7).

Additions of inorganic orthophosphate to macerated mycelial preparations (Table 10) resulted in an initial slight depression of endogenous and exogenous activities followed by sharp temporary stimulation. The duration of the depressive effect was longer with exogenous activity. This behavior with phosphate suggests a partially disorganized system undergoing autolysis analogous to that of yeast cells in contrast to an actively respiring maceration juice preparation (74). Further support in this direction may be derived from the nearly complete suppression of endo-

genous and exogenous activities by toluene either in the presence or absence of added inorganic phosphate (Table 10).

6. *Isolation of methylglyoxal, pyruvic acid, and acetaldehyde using macerated mycelial preparations of Chaetomium funicola.*

In a further endeavor to ascribe the functional significance of phosphorus in the mycelium of *C. funicola* to the phenomenon of phosphorylation in carbohydrate metabolism, repeated attempts were made to isolate phosphoglyceric acid, but these proved unsuccessful. Again in these instances use was made of macerated mycelial preparations obtained immediately before use from freshly harvested young (6-day-old) or old (14-day-old) mycelium or from harvested washed mycelium that had stood at 33°F. for several days. Alterations in certain conditions as time of incubation (up to 22 hours), temperature and the omission, concentration, and type of various substances pointed out by Stone (99) to be influential on phosphoglyceric acid formation in no way influenced the final result. Likewise, the course of phosphorus changes in the liquid portion of these experimental mixtures revealed no removal of inorganic phosphorus but rather a liberation similar to that already described. With these failures attention was then directed toward isolation of methylglyoxal, pyruvic acid, and acetaldehyde.

Preliminary experiments with macerated mycelial preparations from freshly harvested mycelium developed for 12 days in culture, or from a portion of it dried in vacuo over concentrated H_2SO_4 for several days, or another portion stored moist at 33°F. for 2 weeks yielded, in the presence of either hexosediphosphate or glucose with phosphate buffer (pH 6.87) and toluene, derivatives of 2:4-dinitrophenylhydrazine which on solvent fractionation (95) gave colors with alcoholic KOH characteristic for methylglyoxal, pyruvic acid and acetaldehyde and corresponding approximate melting points. In one trial methylglyoxal was found in nearly twice the quantity from hexosediphosphate as from glucose alone. Another trial was then made to obtain sufficient 2:4-dinitrophenylhydrazine derivatives to enable greater purification of the fractions. Five identical mixtures in each of five flasks were prepared with 3.3 gm. moist mycelium, 10 cc. 20 per cent glucose solution, 5 cc. phosphate buffer (pH 6.87), 25 cc. water, and 1 cc. toluene. After 48 hours' incubation, trichloroacetic acid was added to make a 5 per cent concentration, filtered, and a solution of 2:4-dinitrophenylhydrazine added to the filtrate. Precipitation of 2:4-dinitrophenylhydrazine derivatives was allowed to proceed for 3 days at which time they were combined and fractionated. The results obtained were as follows:

Fraction	Recrystallization	Melting point	Color with alcoholic
	medium		KOH
methylglyoxal	nitrobenzene	295°C.	blue violet
pyruvic acid	ethanol	211-214°C.	reddish brown
acetaldehyde	ethanol	167°C.	reddish brown

No mixed melting points were determined because the amount of ma-

terial obtained was small. Simon and Neuberg (95) record the same color reactions for these derivatives but with the following melting points: methylglyoxal, 298°C.; pyruvic acid, 216°C., and acetaldehyde, 164–165°C. Campbell (13) found 168°C. for acetaldehyde and 218°C. for pyruvic acid hydrazones.

DISCUSSION

As previously demonstrated (93) *Chaetomium funicola* converts the carbon of the glucose in Czapek-Dox medium mainly to carbon dioxide and mycelium. In consequence, it continues to form mycelium so long as there is sufficient undecomposed glucose remaining in the medium (94). The removal of phosphorus from the medium accordingly continues as mycelium is formed over a long period of time. In this respect *C. funicola* differs from *Aspergillus niger* but apparently is similar to *Oidium lactis* and *Dematium pullulans* (92).

The very high values for phosphorus in the mycelium of *Chaetomium funicola* grown on media with high initial concentrations of KH_2PO_4 correspond to similar high values reported for *Aspergillus niger*. The greater dissimilation of glucose per unit weight of mycelium found at the higher initial concentrations of KH_2PO_4 is considered due to the retarding influence of high phosphate concentrations on mycelium formation.

The origin of the non-orthophosphate fraction with its components of labile, easily and difficultly hydrolyzable organic phosphorus groups in the media of developing cultures of *C. funicola* may be identified with their liberation from ageing autolyzing hyphae. Schnücke (92) ascribed the apparent existence of an organic phosphorus fraction in the medium of *Aspergillus niger* cultures to errors in the methods of phosphorus analyses, since small negative and positive values were obtained. Braun and Frey (9) found organic phosphorus present in 27-day-old cultures of *A. niger* only on such media as showed an abundance of inorganic phosphorus remaining within the medium. More organic phosphorus (15–45 per cent of the total phosphorus present in the medium) was found in media initially supplied with organic forms of either or both nitrogen (as asparagin and peptone) and phosphorus (as phytin) than with inorganic forms of both. In the latter instances the organic phosphorus formed comprised only 4 per cent of the total phosphorus in the medium. Michel-Durand (69) and Mann (67) found organic phosphorus (2–28 per cent of the total phosphorus) in the medium only after the onset of autolysis, but the amount present was extremely variable and was explained in part by Michel-Durand by the presence of conidia of the fungus in the solutions being analyzed.

The findings of various acid-soluble phosphorus constituents in the mycelium of *Chaetomium funicola* and the other fungi here studied is in accordance with similar findings with yeasts (60, 61, 65), bacteria (79, 116), higher plants (34, 35, 115) and various animal tissues (76, 31, 60, 61, 62). While Vorbrodt and Michel-Durand considered only mineral and organic phosphorus fractions for *Aspergillus niger*, the present study has revealed the presence of inorganic orthophosphate P, labile phosphorus

hydrolyzable in 7 minutes at 100°C. in N HCl, and organic phosphorus of one or more components, one or more resistant to acid hydrolysis and another readily hydrolyzable by acid. Extremely labile phosphorus compounds of the type represented by phosphagen were not detected. The present results do not support Mann's observations (67) that the acid-soluble phosphorus in the mycelium is exclusively of the readily hydrolyzable type.

The significance of the phosphorus fractions in the mycelium of fungi has been variously construed. Koch and Reed (45) and Goupil (30) considered protein phosphorus and lecithin phosphorus to be the most important phosphorus constituents in the mycelia of *A. niger* and *Amylomyces rouxii*. Goupil considered mineral phosphorus in the mycelium to be the product of organic phosphorus degradation while Koch and Reed believed mineral phosphorus to be changed to lecithin and protein phosphorus through an intermediate water-soluble phosphorus fraction. A view similar to that of the latter authors was held by Vorbrodt (112) who assigned a transitory role to the acid-soluble organic phosphorus fraction in the development of *Aspergillus niger* and an essential role to mineral phosphorus in the mycelium, without indicating the nature of those roles. As is evident, these concepts deal only with the incorporation of phosphorus into ultimate mycelial structure and give no hint of the respiratory role that has since emerged in living yeasts, bacteria, and animal cells. In these latter developments, the acid-soluble phosphorus fraction including inorganic orthophosphate P, labile phosphorus including pyrophosphate P, and organic phosphorus such as hexosephosphates and their various degradation products have been demonstrated to form an integral part of carbohydrate dissimilatory processes occurring within the cells in a manner comparable if not identical to the phenomenon of phosphorylation obtained with artificial preparations.

A similar possibility arises for the fungi here studied, but can be determined only through identification of the acid-soluble phosphorus constituents and through a study of their interrelationships under conditions similar to those obtained with yeasts (18, 50, 55, 65, 66, 70, 86) and bacteria (5, 79, 116). The suggestion by Nord *et al.* (76, 77, 78) that glucose dissimilation by *Fusarium* spp. need not go by way of phosphorylation has been criticized (65) on the ground that consideration was given only to the phosphorus in the medium rather than that in the mycelium (102), to which also might be added the anaerobic conditions prevailing under the experiment with consequent phosphorus mineralization (67).

The attempt in the present work to determine the metabolic significance of phosphorus through following the changes in the acid-soluble phosphorus constituents in macerated mycelial preparations proved unsuccessful because phosphorus mineralization was the predominating if not the exclusive reaction. This same general behavior of phosphorus mineralization was obtained under addition of various substances such as glucose, NaF, toluene, KCN, ethyl urethane, iodoacetate and phlorhizin which have proven so successful in similar studies with blood cells (31), yeast, and bacteria. The conclusion may be drawn that conditions were

those of inhibited respiratory activity obtained through inadequate aeration.

The observed mineralization of phosphorus and changes in the acid-soluble phosphorus constituents perhaps finds its counterpart in a similar behavior of phosphorus on autolysis of other cells such as those of muscle (23), liver (2, 26, 90, 100), "magenmucosa," spleen, kidney, pancreas, heart and striated muscle (108), brain (1, 28), blood (89), yeast (61), and bacteria (116). Fungi have scarcely been investigated (33, 40). The increases by approximately 20 per cent of the acid-soluble phosphorus presumably had their origin in either or both the lipid phosphorus fraction (69, 108) or the nuclein phosphorus (33, 40). Such increases were slightly greater and more rapid in the presence of toluene and lower and less rapid in the presence of NaF. Glucose enhanced these increases under both treatments. Inorganic orthophosphate showed ultimate increases of approximately 50-200 per cent which would indicate that by far the greatest mineralization of phosphorus occurred within the acid-soluble phosphorus fraction. The observed increases in amount of organic-phosphorus fractions equal to or smaller than the increases in total phosphorus from non-acid soluble phosphorus fraction would lend support to the concept that the latter fraction was mineralized directly and by way of conversion into some acid-soluble organic phosphorus compound. The identity of the latter with the organic phosphorus resistant to 3-hour acid hydrolysis is suggested by the observed initial increase in this fraction and its subsequent slow decrease during progressive autolysis. The comparative persistence of the labile phosphorus fraction and the more rapid and nearly complete disappearance of the 3-hour acid-hydrolyzable phosphorus fraction would suggest the intermediate role of the former in the dephosphorylation (and possible phosphorylation) of the latter (90).

The metabolic behavior (CO_2 evolution and O_2 consumption) of macerated mycelial preparations of *Chaetomium funicola* is in respect to endogenous activity analogous to that observed for yeast (98) and "*Fusarium* sp. H" (29). Exogenous activity is similar as to sensitivity to NaF and iodoacetate but different in its sensitivity to cyanide and in the R. Q. values which are characteristic for respiration. These findings and others⁷ suggest that exogenous activity with macerated mycelial preparations of *Chaetomium funicola* is fermentative as well as respiratory, probably in accordance with the Pfeffer-Blackman hypothesis (110). Of interest in this connection are the recent views of Runnstrom, Borei and Sperber (91), and Borei (8) who considered the inhibitory effect of NaF to be concerned directly with the respiratory mechanism rather than with the interruption of a primary fermentation (the anaerobic phase of respiration).

⁷ In culture experiments spores of *C. funicola* failed to germinate in a nutrient medium containing iodoacetate in concentrations of M/1,000 or greater while a 50 per cent reduction in growth occurred with M/10,000 iodoacetate. Sodium fluoride M/500 caused a 75 per cent reduction in growth, and higher concentrations of NaF caused further reductions in growth till complete inhibition was obtained with M/10 NaF.

The observed induction period and subsequent rise in rates of metabolic activity of macerated mycelial preparations of *C. funicola* suggest a partially disorganized system which is undergoing autolysis (74, 75).

The failure in the present study to isolate phosphoglyceric acid and to detect any significant decrease in inorganic orthophosphate concentration in the experimental medium cannot be taken as direct evidence against the concept of phosphorylation as a process occurring in the carbohydrate metabolism of *C. funicola* (19). The manner of treatment of the mycelium may have been too drastic and age of the mycelium may be an important consideration also (29, 32, 36, 102, 106). The demonstration of methylglyoxal, pyruvic acid, and acetaldehyde in the present work also cannot be considered evidence against the phosphorylation scheme but may even favor it since their origin may reside with some phosphorylated intermediate product (80).

In the present study recourse was made to the use of macerated mycelial preparations with the purpose of subdividing and macerating the mycelial hyphae to obtain a more uniform distribution of the material throughout the experimental medium. Although grinding with sand had destroyed some of the mycelial fragments, microscopic examination revealed the presence of considerable quantity of intact hyphae. Tamiya (104) used a similar preparation to determine the dehydrase activity of *Aspergillus oryzae*. Bernhauer and Wolf (6) used *A. niger* mycelium macerated in a mortar with pestle under addition of CaCO_3 and obtained the formation of gluconic and oxalic acids in the presence of added glucose. More recently Gould and Tytell (29) successfully used young hyphal materials of "*Fusarium* sp. H" that had been dispersed by shaking with glass beads in a phosphate buffer solution and likewise older mycelia that had been finely minced before shaking with glass beads for dispersion. Earlier investigators of mycelial respiratory activity used carefully teased-out mycelial hyphae (32), intact, uninjured mycelial mats (46, 47, 85, 102, 103), variously dried preparations (37, 48, 49), or press juice (42, 71).

Three species of *Fusarium* and *Aspergillus niger* were included with *Chaetomium funicola* in the present study to obtain a range of fungi differing essentially in their biochemical properties as revealed by the products accumulated. Thus, while *C. funicola* converts glucose mainly to carbon dioxide and mycelium, *Aspergillus niger* produces such acids as oxalic, citric, and gluconic; and *Fusarium* spp. are known to produce ethanol in fair amounts. The inclusion of *Aspergillus niger* was of interest because of its ability to form gluconic acid directly from glucose by the glucoseoxidase enzyme without the necessary participation of phosphorus, and because of the disputed (10) Chrzaszcz-Tiukow hypothesis (4, 14, 105) underlying the formation of oxalic, citric, and other acids from glucose, which assumes an initial anaerobic phase of glucose dissimilation identical to alcoholic fermentation. *Fusarium* spp. were included because the mechanism underlying their formation of alcohol has been assumed to be similar to that of yeasts (29) except that Nord (76, 77, 78) has suggested that glucose dissimilation by some *Fusarium* spp. need not go by way of phosphorylation.

SUMMARY

1. The phosphorus relations of *Chaetomium funicola* were studied with cultures developing on Czapek-Dox medium. The depletion of phosphorus in the medium was followed through inorganic orthophosphate P and total phosphorus analysis at several intervals in the development of the fungus. Analysis was made of the acid-soluble phosphorus constituents in the mycelium of *C. funicola* and four other fungi. An interpretation of the significance of these acid-soluble phosphorus constituents in the mycelium was sought in the light of the phenomenon of phosphorylation applied to carbohydrate dissimilation.

2. *Chaetomium funicola* continued to develop and remove phosphorus from the medium throughout the studied period of 23 days' development. Mycelial development was not stopped with depletion of the inorganic orthophosphate supply in the medium as obtained with one-fourth normal initial concentrations of KH_2PO_4 in the medium, but continued presumably in a similar manner to that which allowed for mycelial development in the media with no additions of KH_2PO_4 .

3. Greater removal of phosphorus from the medium occurred with higher initial concentrations of phosphorus, yielding correspondingly higher total and percentage phosphorus contents in the mycelium.

4. Varying the initial pH of the medium did not influence the removal of phosphorus from the medium except under alkaline and very acid conditions where the amount of mycelium formed and phosphorus removed was retarded.

5. A non-orthophosphate P fraction in the medium was detected in amounts of 20 per cent of the total phosphorus remaining in the medium at 10 and 23 days' development of the fungus. Analysis showed this fraction to contain labile phosphorus and organic phosphorus of a type resistant to hydrolysis. Its presence was presumed due to its liberation from ageing or autolyzing hyphae.

6. Inorganic orthophosphate P, labile phosphorus, and organic phosphorus of the difficultly hydrolyzable type were found in the acid-soluble constituent of mycelia of *C. funicola*, *Aspergillus niger*, *Fusarium lini*, *F. bulbigenum* var. *niveum*, and *F. oxysporum* var. *cubense*.

7. Comparison of the acid-soluble phosphorus constituents in mycelium from 8- and 18-day-old cultures of *Chaetomium funicola* revealed a lower percentage of inorganic orthophosphate P and a higher percentage of labile phosphorus in the older mycelium. Indications were obtained in both mycelia that the organic phosphorus was composed of two parts, one resistant to acid hydrolysis and the other quite susceptible.

8. The significance of the acid-soluble phosphorus fractions in *C. funicola* and the other four fungi was considered in the light of recent investigations on carbohydrate metabolism with living yeasts, bacteria, and animal cells, and was investigated particularly with *C. funicola* for a similar interpretation.

9. Incubation of macerated mycelial preparations of *C. funicola* and *Fusarium* spp. at 30°C. with a view to determining the interrelationship

of the different acid-soluble phosphorus fractions in the mycelium in a manner similar to that followed for living yeasts, bacteria, and blood cells revealed autolytic mineralization of phosphorus to be the predominating reaction of such preparations under the conditions employed. Maximal increases of 10–27 per cent in total acid-soluble phosphorus and 50–200 per cent in inorganic orthophosphate P were obtained within a period of approximately 18 hours. Labile phosphorus and 3-hour acid-hydrolyzable organic phosphorus generally decreased in amount with progressive autolysis, with the decrease of the latter being more rapid and more complete than that of the former. Organic phosphorus resistant to 3-hour acid hydrolysis showed initial increases with subsequent decreases suggesting its origin to reside with some acid-insoluble fraction. Sodium fluoride M/30 retarded while toluene accelerated these changes.

10. A determination of the respiratory activity of macerated mycelial preparations of *C. funicola* in a Warburg-Barcroft respirometer revealed endogenous and exogenous activities of these preparations with the former activity being characteristically oxidative and the latter suggestive of respiration with an initial fermentative phase. Evidence obtained suggested that these preparations were partially disorganized and undergoing autolysis.

11. Direct attempts toward demonstrating phosphorylation as a significant process in carbohydrate metabolism of *C. funicola* using macerated mycelial preparations showed neither phosphoglyceric acid formation nor phosphorus uptake. Methylglyoxal, pyruvic acid, and acetaldehyde formation, however, was readily demonstrated.

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HYGROSCOPICITY, PROPERTIES IN BORIC ACID SOLUTION, AND SPECIFIC VISCOSITIES OF MIXTURES OF THE DIASTEREOMERIC 2,3-BUTANEDIOLS¹

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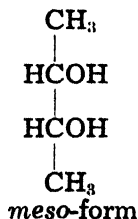
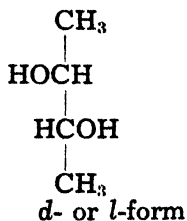
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2,3-butanediol, commonly called 2,3-butylene glycol, has also been named as β -butylene glycol, symmetrical butylene glycol, 2,3-dihydroxybutane, β,γ -dihydroxybutane, symmetrical dimethyl ethylene glycol, and pseudo butylene glycol. The glycols are generally classified as aliphatic compounds whose formulas contain two hydroxyl groups. Aldehydes and ketones give derivatives, such as the acetals and the hemiacetals, containing two substituted hydroxyl groups, but such compounds are not classed as glycols.

The general properties and methods of preparation of the glycols have been discussed in detail by Lawrie (12). Over 100 glycols are known but, until recently, ethylene glycol and pinacol were the only ones to assume importance. The boiling points of the glycols range upward from 180° C., but there is no correlation between the boiling points and molecular weights. The glycols are very hygroscopic, and it is often difficult to remove the last traces of water from them. A summary of some of the most important properties of the glycols has been given by Lees (13).

The glycols of the general formula $R \cdot \text{CHOH} \cdot \text{CHOH} \cdot R$ exist in two diastereoisomeric forms. These forms for 2,3-butanediol are:



HISTORICAL

Harden and Walpole (9) were the first to show the production of acetylmethylcarbinol and 2,3-butylene glycol by the action of bacteria on sugars. They found that 27 per cent of the dextrose fermented by

¹ This work was supported by a grant from the Industrial Science Research Institute of the Iowa State College for studies on the fermentative utilization of agricultural products. The paper is based on the thesis presented in 1942 by Mr. T. M. Lees for the degree of Master of Science. Publication has been delayed by a War Department secrecy order forbidding publication of information in regard to l-2,3-butylene glycol. The secrecy order was lifted as of November 29, 1943.

B. lactis aerogenes, under anaerobic conditions, was converted to 2,3-butylene glycol. Fulmer, Christensen, and Kendall (7) determined optimum conditions for the production of 2,3-butylene glycol by the action of four species of *Aerobacter* on sucrose in a synthetic medium. While there were no great differences in glycol yields among the organisms tested, *Aerobacter aerogenes* was found to be the most satisfactory for general use. Under optimum conditions 98 per cent of the sugar was fermented of which 47 per cent was converted to 2,3-butylene glycol; this yield is about 90 per cent of theory. The authors also presented a brief review of previous work on the fermentative production of 2,3-butylene glycol.

Walpole (15) was one of the first to investigate the nature of the isomers present in fermentation 2,3-butylene glycol. He obtained, by fractionation, two phenylurethane derivatives melting at 199.5° and 157° C., respectively. The former constituted about 90 per cent of the glycol. While he did not commit himself as to the nature of the predominating compound, later information indicates that it was the *meso*-glycol. Böeseken and Cohen (3) decided, on the basis of resolution data, that the *meso*- form predominates in fermentation 2,3-butylene glycol.

The best method heretofore available for the analysis of mixtures of the *meso*- and *dl*-glycol is based upon melting point data presented by Wilson and Lucas (17); the glycols were prepared synthetically. They gave the melting points of the *meso*- and *dl*-glycols as 34.4° and 7.6°, respectively. The melting point-composition curve shows an eutectic point, by extrapolation, at about -15° C. and about 40 per cent of *meso*-glycol content. The melting points of mixtures containing less than 30 per cent of the *meso*- form are difficult to determine. The hygroscopicity of the glycol, its tendency to form supercooled solutions, and the increased viscosity at low temperatures all contribute to making the melting point difficult to determine near the eutectic point. It should also be noted that, in the temperature range of 7.6° to -15° C., the melting point is a two-valued function of the composition, that is, there are two combinations of the glycols with identical melting points.

There is need for the development of more adequate methods for the determination of the proportions of the glycols in mixtures. The data presented in this paper involve mixtures of the *meso*- and *l*-2,3-butylene glycols. The *d*-2,3-butylene glycol should have the same physical constants and behavior as the *l*-form except as to sign of optical rotation.

MATERIALS

The 2,3-butylene glycol used as the source of the *meso*-glycol employed in obtaining the data presented below was obtained by the action of *Aerobacter aerogenes* upon dextrose. The melting point showed the glycol to contain 90 per cent of the *meso*- form. Fulmer, Underkoffler, and Bantz (8) studied the production of acetylmethylcarbinol by the action of *Acetobacter suboxydans* upon the 2,3-butylene glycol described above. The yield of carbinol was about 90 per cent of theory. The unfermented

glycol was recovered and purified. The angle of rotation was $[\alpha]_D^{25} + 10.15$. It was concluded from these studies that the organism attacked the *meso*-glycol preferentially and that the original glycol consisted of *meso*- and *d*-glycol with little or no *l*-glycol.

The *meso*-glycol used in subsequent experiments was prepared as follows. The fermentation glycol, containing 90 per cent of the *meso*-form, was subjected to vacuum distillation through a Widmer column with an inner core 16 inches in length. Fractions were removed by means of an all-glass fraction cutter. The pressure was from 1 to 2 mm. of mercury; the rate of distillation was approximately 1 drop each 4 seconds. The fraction used for further purification boiled at 180° C. (729.2 mm.).

Fractional crystallization, using isopropyl ether as the solvent (17), was employed for isolation of the *meso*-2,3-butylene glycol. About 400 ml. of the fraction, prepared as above described, was dissolved in 600 ml. of freshly distilled isopropyl ether. The all-glass apparatus consisted of a 2-liter, long-necked boiling flask closed with a calcium chloride tube. The mixture was cooled in an ice bath and left for half an hour with occasional shaking. The ether was then carefully decanted from the porous mass of crystalline *meso*-glycol. Another portion of 600 ml. of isopropyl ether was added and the crystallization repeated. In all, six recrystallizations were made. The flask containing the last fraction was evacuated over night. The glycol was then melted and quickly transferred to a round-bottomed flask and subjected to distillation through an insulated Vigreux column. After the ether had distilled, the glycol came over at 181° C. The distillate was redistilled through the same column, about 100 g. of the final distillate being obtained. The melting point determination showed this final fraction to contain at least 99 per cent of the *meso*-glycol. The angle of rotation was zero.

The *l*-2,3-butylene glycol was obtained through the courtesy of Dr. R. D. Coghill of the Northern Regional Research Laboratory. The material was redistilled through an insulated Vigreux column. The angle of rotation was $[\alpha]_D^{25} - 13.0$. In absence of evidence to the contrary, this glycol was assumed to be the pure *l*-2,3-butylene glycol. If this assumption should later prove to be incorrect some of the results given below would need to be recalculated, but the basic principles established would still hold.

Each type of glycol was dispensed from a separate 50 ml. burette equipped with a calcium chloride tube at the top and a removable calcium chloride tube at the tip.

EXPERIMENTAL

1. The Hygroscopicity of 2,3-Butylene Glycol

It was previously noted that the glycols are very hygroscopic. Data are given in Table 1 showing the hygroscopicity of 2,3-butylene glycol. The glycol used, produced by the action of *Aerobacter aerogenes*, was the same material as that used for the separation of the *meso*-glycol. A 17.7 g.

sample of the glycol, in a crystallizing dish, was placed in a desiccator containing water and left at room temperature which did not vary greatly from 25° C. The depth of the glycol was about 1 cm. and the surface-volume ratio was 1.07. The dish and contents were weighed periodically

TABLE 1
HYGROSCOPICITY OF 2,3-BUTYLENE GLYCOL

Time, Hours	W *		Time, Hours	W *	
	Exper.	Calc. from Eq. (1)		Exper.	Calc. from Eq. (1)
4	3.2	4.0	263	62.0	61.7
16	10.1	10.0	306	69.1	68.1
29	14.3	14.7	342	74.7	73.1
41	18.6	18.4	380	79.9	78.3
53	21.8	21.7	428	86.4	84.7
66	25.3	25.1	474	92.4	90.6
90	30.8	30.8	534	99.4	98.0
116	35.7	36.3	602	106.8	105.7
163	44.4	45.2	672	113.3	113.8
190	49.5	50.0	784	125.6	125.3

* W = grams of water absorbed per 100 grams of glycol.

to determine the weight of water absorbed. The percentage of water absorbed (grams of water absorbed per 100 grams of glycol), w , proved to be a parabolic function of the time, t , the equation being:

$$(1) \log w = 0.651 \log t + 0.216.$$

Calculated values, using this equation, are given in Table 1.

These data emphasize the importance of care in keeping moisture from glycol samples. The occasional abnormally large deviation of experimental values from the theoretical, shown by data in subsequent experimental results, may be attributed in large measure to accidental entrance of moisture into the prepared samples of the glycols.

2. The Specific Viscosities of Mixtures of Meso-2,3-Butanediol and l-2,3-Butanediol

Data in Table 2 show the specific viscosities of mixtures of the two glycols. The apparatus consisted simply of an ordinary 5 ml. pipette with an added graduation below the bulb. The determinations were made at 30° C. The time required for the mixture to flow from one graduation to another was measured by means of a stop-watch. The ratio of the drainage times for the mixtures to that for water is the specific viscosity. The densities of the two glycols are so near to each other and to that of water that this simple relation is justified.

The specific viscosity, s , proved to be an exponential function of the

percentage of *meso*-glycol of the type $\log(y - c) = mx + b$, from which was derived the equation,

$$(2) \quad \% \text{ meso-glycol} = 140.0 \log(s - 2.87) - 55.2.$$

Values calculated by this equation are given in Table 2.

The specific viscosity method is simple and satisfactory for the determination of mixtures of the glycols. However, it should again be empha-

TABLE 2
SPECIFIC VISCOSITIES OF MIXTURES OF *meso*-2,3-BUTANEDIOL AND *l*-2,3-BUTANEDIOL

t^*	s^\dagger	Per Cent <i>meso</i> -Glycol	
		Experimental	Calc. from Eq. (2)
44.3	5.34	0	0
48.7	5.88	16.6	11.8
58.6	7.07	33.8	32.0
70.6	8.52	50.1	50.1
85.5	10.32	66.9	66.9
106.4	12.83	83.4	84.5
130.4	15.72	100.0	100.0
8.3 (water)			

* s = specific viscosity.

† t = drainage time in seconds.

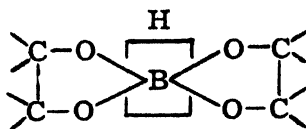
sized that extreme care must be taken to insure against taking up of moisture by the samples.

3. Specific Conductivities and pH Values of Mixtures of *Meso*-2,3-Butanediol and *l*-2,3-Butanediol

It is well known that certain polyhydric compounds react with boric acid, in solution, to form complex acids which are strong electrolytes. For example, boric acid can be titrated as a monobasic acid in the presence of glycerol or mannitol using phenolphthalein as indicator. Magnanini (14) was one of the first to apply conductometric methods to polyhydric compounds in boric acid solution. Böeseken and coworkers (1, 2, 3, 4, 5, 6) extended the earlier work with a primary interest in the stereochemical relations of the polyhydric compounds to the phenomenon. The methods developed by them have proved useful in determining the configurations of certain of the sugars.

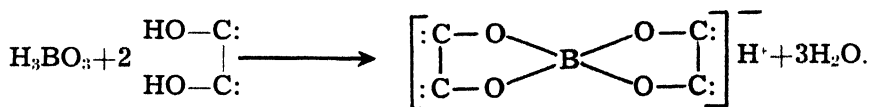
The increase in electrical conductivity, with accompanying decrease in pH, of the polyhydroxy compound in boric acid solution is associated with the formation of a more highly ionizable complex of the two compounds. Conditions are most favorable to the formation of the complex when the two hydroxyl groups are on adjacent carbon atoms and lie on the same side of the carbon atoms. Hermans (10) assigned the follow-

ing structure to the complex:



That is, two molecules of the polyhydroxy compound combine with one molecule of the boric acid.

Böeseken (6) confirmed the results of Hermans and wrote the reaction as follows:



Whitmore (16) described the complex as follows: "The complex has the tetrahedral grouping of five atoms and 32 electrons which characterizes the ions of H_2SO_4 , H_3PO_4 , and HClO_4 . Similar products are formed with arsenious acid. Whenever two hydroxyls on adjacent carbons can form this tetrahedral arrangement with a boron or an arsenic atom the conductivity is increased. The only thing which can prevent this union is the location of the two adjacent hydroxyls on opposite sides of the plane of a ring compound such as the furanose and pyranose forms of sugars." It may well be that with higher glycols hindered rotation about a single bond will have the same effect.

Böeseken and Cohen (3) have studied the configurations of the 2,3-butylene glycols by the boric acid method. They used a glycol obtained by the fermentative action of *Aerobacter aerogenes* and a *dl*-glycol obtained by synthesis. They concluded that the hydroxyl groups of both samples of glycol were in an unfavorable position to form the boric acid complex.

Previous reports in the literature on this subject are so meager and inconclusive that further work seemed desirable. The authors present data in the present communication which show that the *meso*- and *l*-2,3-butylene glycols do differ in respect to properties in boric acid solution to a sufficient degree to form the basis for analytical methods for the determination of the composition of mixtures of the glycols.

The conductivity apparatus was a simple setup employing a Leeds and Northrup Student Potentiometer, two decade boxes, a 1,000-cycle audio-oscillator, and head phones. The cell used was of the Freas type with platinized electrodes. All determinations were made at 25° C. The specific conductivity was calculated by the relation $L = K/R$, in which L is the specific conductivity, K is the cell constant and R is the resistance of the solution in ohms. The pH values were determined with a Cameron glass electrode apparatus.

a. *Specific conductivities.* Preliminary experiments were made varying the glycol and boric acids separately to determine the best conditions for the standardization of the method. In the standard conditions chosen, each sample contained 5.00 g. of the mixture of glycols diluted to 50.0 ml. with 0.5 M boric acid solution. A maximum conductivity was obtained with 9.00 g. of glycol mixture in 0.5 M boric acid solution. However, the supply of glycol was limited, and the difference between the conductivities with 5.00 g. and 9.00 g. of glycol was not sufficient to warrant using the larger amount. The conductivities and pH values were determined after the mixtures had been allowed to stand for 48 hours to assure stable conductivity values.

The specific conductivity data are given in Table 3. It is evident that the specific conductivity decreases markedly with increase in content of

TABLE 3
SPECIFIC CONDUCTIVITIES OF MIXTURES OF *meso*-2,3-BUTANEDIOL AND *l*-2,3-BUTANEDIOL

* $L \times 10^5$	Per Cent <i>meso</i> -Glycol	
	Experimental	Calc. from Eq. (3)
6.91	0	-0.6
6.13	16.6	16.6
5.28	33.8	35.6
4.61	50.1	50.6
3.88	66.9	67.0
3.13	83.4	83.7
2.49	100.0	98.1

* L = specific conductivity.

meso-glycol. The specific conductivity is a linear function of the composition, the equation being:

$$(3) \quad \% \text{ meso-glycol} = 153.6 - (22.3 \times L \times 10^5).$$

Calculated values, using this equation, are given in Table 3. The specific conductivity of the standard fermentation glycol was 2.96×10^5 . The calculated percentage of *meso*-glycol, using equation (3), was 88 per cent. This value agrees with that of 90 per cent obtained by the melting point method.

b. *pH values.* The pH values of the glycol mixtures, in 0.5 M boric acid solution, are given in Table 4. As would be expected from the conductivity data, the pH increased with increased content of the *meso*-glycol. A mathematical analysis of the data showed the hydrogen ion activity to be a linear function of the composition. The hydrogen ion activity is defined as $a_{H^+} = \text{antilog} (-\text{pH})$. From this relation the following equation was derived:

$$(4) \quad \% \text{ meso-glycol} = 123.6 - (4.81 \times a_{H^+} \times 10^5).$$

Calculations using this equation are given in Table 4.

It is evident that the change in pH values is not as wide as the change in specific conductivity. The former offers the advantage of general availability of equipment for measuring pH. While traces of water

TABLE 4
pH VALUES OF MIXTURES OF *meso*-2,3-BUTANEDIOL AND *l*-2,3-BUTANEDIOL

pH	Per Cent <i>meso</i> -Glycol	
	Experimental	Calc. from Eq. (4)
3.59	0	0
3.65	16.6	15.9
3.71	33.8	29.8
3.82	50.1	51.0
3.93	66.9	66.8
4.08	83.4	83.6
4.31	100.0	100.0

are not as serious in these determinations as with specific viscosity determinations, small traces of ionized materials should be carefully avoided.

SUMMARY

1. Detailed procedures have been described for the isolation of *meso*-2,3-butylene glycol from the glycol produced by the action of *Aerobacter aerogenes* upon sugars.

2. Data are presented on the hygroscopicity of 2,3-butylene glycol. The glycol proved to be highly hygroscopic. The water absorbed per 100 g. of glycol, in an atmosphere saturated with water vapor at about 25° C., is a parabolic function of the time of exposure.

3. The specific viscosity of mixtures of *meso*- and *l*-2,3-butylene glycols is an exponential function of the composition of the type $\log(y-c) = mx + b$. The method developed furnishes a simple and satisfactory procedure for the analysis of mixtures of the glycols.

4. The specific conductivity of mixtures of *meso*- and *l*-2,3-butylene glycols in 0.5 M boric acid solutions is a linear function of the composition. The hydrogen ion activity of the mixtures is a linear function of the composition. There is a wider range of change in conductivities than the change of pH values. Both conductivities and pH values in boric acid solutions furnish procedures for the analysis of mixtures of the two glycols.

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THE USE OF ALFALFA EXTRACT TO SUPPLY NUTRIENTS FOR THE GROWTH AND CHEMICAL ACTIVITIES OF *ACETOBACTER SUBOXYDANS*¹

E. I. FULMER, A. C. BANTZ, AND L. A. UNDERKOFER

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One of the projects conducted under the Elastomer Program of the Iowa State College is concerned with studies on the fermentative production of chemicals which might be employed as intermediates in the preparation of plastics, particularly those of the elastomer type. One such chemical is acetylmethylcarbinol which has hitherto been available only in very small amounts. It has recently been reported from these laboratories (4) that acetylmethylcarbinol may be produced in excellent yields by the action of *Acetobacter suboxydans* upon 2,3-butylene glycol. Yeast extract was used to furnish nutrients for the organism in the laboratory study of this fermentation.

Yeast extract has been employed in the numerous previous investigations reported from these and other laboratories on the use of *A. suboxydans* to convert polyhydric alcohols to the corresponding keto-compounds, such as the production of *l*-adonose from adonitol (6), *l*-sorbitose from sorbitol (2), dihydroxyacetone from glycerol (5, 11), *l*-erythrulose from erythritol (14), levulose from mannitol (3), perseulose from perseitol (9), and acetylmethylcarbinol from 2,3-butylene glycol (4). The growth requirements of *A. suboxydans* have recently been ascertained by Underkofler, Bantz, and Peterson (10) who found that for satisfactory growth of the organism the medium must contain a suitable carbon source, organic nitrogen, mineral salts, pantothenic acid, *p*-aminobenzoic acid, and nicotinic acid.

Since, for large-scale operations, the use of the pure nutrient materials is out of the question, and yeast extract is also a relatively expensive material, a cheaper source of nutrients for the organism is desirable. Along this line Wells, Stubbs, Lockwood, and Roe (13) and Stubbs, Lockwood, Roe, Tabenkin, and Ward (7) reported that corn steep liquor served to supply adequate nutrients for the semi-commercial production of *l*-sorbitose from sorbitol and for the production of 2-ketogluconic acid from glucose by *A. suboxydans*.

A readily available material known to be a rich source of vitamins and other growth-promoting substances is alfalfa. Workers in these laboratories used alfalfa extracts as a source of "bios" in the early study of the growth requirements of yeast. Subsequent work in many labora-

¹ This work was supported by a grant from the Industrial Science Research Institute of the Iowa State College for studies on the fermentative utilization of agricultural products.

tories has shown that "bios" is not a single substance. The earliest recognition of this fact was by Fulmer, Duecker, and Nelson (1) who demonstrated that more than one factor was involved in the stimulation of yeast growth by alfalfa extracts. More recent work in many laboratories has determined the stimulating effect of a number of pure chemical compounds, as well as some of still unknown constitution upon the growth of yeast. This work has been surveyed by Williams (15). Other investigators who have reported the presence of growth substances in alfalfa are Williams and Christensen (16) and Tatum, Peterson, and Fred (8). It seemed highly probable that alfalfa extracts might contain the necessary growth factors for *A. suboxydans*. The present paper deals with the use of various extracts of alfalfa to support the growth of *A. suboxydans* and to enable this organism to bring about the desired conversion of the polyhydric alcohols to the keto-compounds.

EXPERIMENTAL

CULTURE AND MEDIA

The culture of *Acetobacter suboxydans* was originally obtained from the American Type Culture Collection listed as No. 621. The stock cultures are carried on yeast extract-glycerol-agar slants. The stock culture was transferred to a medium containing 5 per cent glycerol and 0.5 per cent yeast extract (Difco powdered product) and kept active by subculturing on fresh medium each 48 hours. The inoculum for all growth studies was obtained from a 24-hour culture grown on 10 ml. of 5 per cent glycerol—0.5 per cent yeast extract medium in a 50 ml. Erlenmeyer flask. The culture was shaken, transferred aseptically to a sterile test tube plugged with cotton, and centrifuged. The clear liquid was poured off and the cells washed twice with 10 ml. of sterile 0.9 per cent saline solution by centrifugation, and finally suspended in 10 ml. of the sterile saline solution. One drop (ca. 0.05 ml.) of this suspension was used for inoculating 10 ml. of test medium in a 50 ml. Erlenmeyer flask. The basal media for all growth studies contained 5 per cent glycerol.

The inoculum for all conversion studies was obtained from a 24-hour culture grown on a medium having the same composition as that to be inoculated. In all cases the organism had been subcultured twice on this same medium. The inoculum consisted of 6 drops of culture per 10 ml. of medium. All fermentations were run in duplicate, and the values reported are the averages for the duplicate determinations. All media were adjusted to pH 6.0 ± 0.1 , which is optimum for the growth of the organism (10), and sterilized for 15 minutes at 15 lbs. steam pressure. The incubation temperature was 28°C.

ANALYTICAL METHODS

Growth was determined quantitatively by measuring turbidity. The 10 ml. of culture was shaken, transferred to a test tube, and the bacterial cells were uniformly suspended by means of a glass homogenizer. Tur-

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bidity was then measured by means of a KWSZ photometer, using a 650 m μ filter. The instrument was first adjusted to read 100 with the uninoculated medium in a photometer tube, the homogenized culture in a photometer tube was then placed in the instrument and the percentage of transmitted light read directly. Thus, there is an inverse relation between the photometer reading and the degree of turbidity (growth) of the culture.

The course of the conversion of the polyhydric alcohols to the corresponding keto-compounds was followed by determining the content of reducing substances developed in the media, using a modified Shaffer-Somogyi method developed in these laboratories (12).

EFFECT OF VARIOUS AQUEOUS EXTRACTS OF ALFALFA ON THE GROWTH OF *A. suboxydans*

Each extract was prepared by mixing 10 g. of alfalfa with 200 ml. of distilled water and then treating the mixture in one of several ways. The various treatments consisted of heating the mixtures at 100°C. for 4 or 24 hours, or at 15 lbs. steam pressure for 15, 30, or 60 minutes. After treatment was complete the mixtures were filtered and the filtrates placed under toluene to prevent contamination by microbial growth. The volume of extract added to the medium in each case was equivalent to adding 0.5 g. of the dried alfalfa meal per 100 ml. of medium. The treatments given the alfalfa-water mixtures and the results of the growth experiments are given in Table 1. It is evident that growth factors were extracted by the various treatments, but in no case did the growth of the organism approach that obtained in the yeast extract control medium.

EFFECT OF VARIOUS ACID EXTRACTS OF ALFALFA ON THE GROWTH OF *A. suboxydans*

The acid extracts were obtained by treating mixtures of 10 g. of alfalfa and 200 ml. of 0.01 or 0.1 N hydrochloric acid by the same methods employed for the aqueous extracts. The mixtures were again filtered and the clear filtrates placed under toluene. The volume of extract added to the medium in each case was equivalent to adding 0.5 g. of alfalfa

TABLE 1
EFFECT OF VARIOUS AQUEOUS EXTRACTS ON THE GROWTH OF *A. suboxydans*

Method of Treating Alfalfa to Produce Extract	Photometer Reading		
	24 hrs.	48 hrs.	96 hrs.
4 hours at 100° C.....	58.4	57.3	48.0
24 hours at 100° C.....	56.4	47.9	41.7
15 min. at 15 lbs. steam pressure.....	60.9	50.7	45.1
30 min. at 15 lbs. steam pressure.....	69.9	64.5	63.2
60 min. at 15 lbs. steam pressure.....	69.2	61.8	60.5
Yeast extract control.....	7.9	6.6	6.2

meal per 100 ml. of medium. The methods of treatment and the results of the growth experiments are given in Table 2. The data show quite conclusively that the extracts obtained with the 0.01 N acid gave better growth in all cases than with the water extracts or with the 0.1 N acid extracts. The greatest growth was obtained with the extract prepared by heating with 0.01 N acid for 24 hours at 100°C. The growth in this case was superior to that obtained using an aqueous extract prepared under the same conditions of heating. Growth was not as good as that

TABLE 2
EFFECT OF VARIOUS ACIDIC EXTRACTS UPON THE GROWTH OF *A. suboxydans*

Method of Treating Alfalfa to Produce Extract	Photometer Reading		
	24 hrs.	48 hrs.	96 hrs.
0.1 N HCl, 4 hrs. at 100° C.....	90.8	88.7	90.0
0.01 N HCl, " " " " ".....	92.7	60.1	48.6
0.1 N HCl, 24 " " " " ".....	93.3	90.0	90.0
0.01 N HCl, " " " " ".....	84.6	42.6	35.3
0.1 N HCl, 15 min. at 15 lbs.....	66.8	65.4	65.0
0.01 N HCl, " " " " ".....	60.9	50.7	45.1
Yeast extract control.....	8.0	7.0	6.3

obtained in the yeast extract control, but the actual amount of alfalfa extract added contained appreciably less solids content than the 0.5 per cent of yeast extract used in the control medium.

EFFECT OF VARIOUS BASIC EXTRACTS OF ALFALFA ON THE GROWTH OF *A. suboxydans*

The basic extracts were prepared by treating mixtures of 10 g. of alfalfa and 200 ml. of 0.01 N sodium hydroxide solution at 100° C. for 4 hours and for 24 hours. Table 3 gives the methods of treatment and the results of the experiments. The basic extracts proved to be of little value as the growth was less than that obtained with the best aqueous or acidic extracts.

PREPARATION OF DRY ALFALFA EXTRACT

The solutions of alfalfa extract were prepared by the methods previously described. In the early experiments the clear filtrate from an

TABLE 3
EFFECT OF VARIOUS BASIC EXTRACTS UPON THE GROWTH OF *A. suboxydans*

Method of Treating Alfalfa to Produce Extract	Photometer Reading		
	24 hrs.	48 hrs.	96 hrs.
0.01 N NaOH, 4 hrs. at 100° C.....	69.9	67.0	60.9
0.01 N NaOH, 24 " " " " ".....	67.9	58.7	55.4
Yeast extract control.....	8.7	6.9	6.7

extraction was subjected to distillation *in vacuo*. When the solution of extract had reached the consistency of a thick syrup, distillation was stopped and the syrup was placed in a vacuum desiccator over calcium chloride. After 24 hours in the desiccator the syrup was treated with 95 per cent ethanol and again placed in the desiccator. The desiccator was evacuated at intervals until a nearly dry material remained. This material was then triturated with absolute ethanol, and again placed in the desiccator which was evacuated periodically until the material was dried. This last step was repeated until the extract had been completely dried. The dried material was then ground using a mortar and pestle. The dried alfalfa extract was used for preparing media in the same manner as powdered yeast extract. In later experiments the vacuum distillation was omitted, and instead the solution of alfalfa extract was evaporated on a steam bath. After a syrup was produced the syrup was treated as described above. There was no difference in the potency of the extracts produced by either method.

The amount of dry extract obtained per gram of alfalfa meal seemed to be nearly constant regardless of the manner of treating the alfalfa. The average value obtained from a large number of experiments was 0.289 g. of dry extract per gram of alfalfa meal, when 10 g. of alfalfa were extracted with 200 ml. of liquid.

Subsequent experiments showed that it is possible to markedly increase the weight of alfalfa meal extracted by the same volume of liquid and still obtain the same relative amount of dry alfalfa extract. For example, when 10, 30, and 40 g. of alfalfa were extracted with 200 ml. of solution, in each case 0.289 g. of dry extract was obtained per gram of alfalfa meal.

EFFECT OF DRY ALFALFA EXTRACTS ON THE GROWTH OF *A. suboxydans*

The media for these experiments contained 5 per cent glycerol, and 0.5 per cent of dry alfalfa extract in each case. This amount of alfalfa extract was used since the amount of yeast extract employed in the control flasks was 0.5 per cent. The results of the growth experiments with the dry extracts are given in Table 4. These data show that the extract

TABLE 4

EFFECT OF DRY ALFALFA EXTRACTS UPON THE GROWTH OF *A. suboxydans*

Method of Treating Alfalfa to Produce Extract	Photometer Reading		
	24 hrs.	48 hrs.	96 hrs.
<i>Aqueous extracts:</i>			
15 min. at 15 lbs. steam pressure.	50.9	31.1	25.0
24 hrs. at 100° C.	39.1	29.4	25.8
<i>Acidic extract, 0.01 N HCl:</i>			
24 hrs. at 100° C.	30.6	24.5	21.7
<i>Basic extract, 0.01 N NaOH:</i>			
24 hrs. at 100° C.	48.3	36.9	32.8
Yeast extract control.	8.4	7.1	6.5

made with 0.01 N hydrochloric acid at 100°C. for 24 hours, was superior to the others tested. The growth was not only more rapid, but there was greater growth in the case of this acidic extract. The growth was not as good as that obtained in the yeast extract control, but the growth obtained with the dry alfalfa extract approached that of the control much more closely than in any previous tests.

EFFECT OF DRY ALFALFA EXTRACT ON THE CONVERSION OF POLYHYDRIC ALCOHOLS TO THE CORRESPONDING KETO-COMPOUNDS

It does not necessarily follow that maximum growth of an organism is required for maximum conversion of substrate. Therefore, even though the growth obtained using alfalfa extract was not quite as good as that obtained with yeast extract it seemed advisable to determine if the growth obtained with alfalfa extract was sufficient to bring about the desired conversions of polyhydric alcohols to the keto-compounds.

In all tests 5 per cent of the polyhydric alcohol was used with either 0.5 per cent yeast extract or 0.5 per cent alfalfa extract. The alfalfa extract used in all cases was the extract obtained by extracting alfalfa meal for 24 hours at 100°C. using 0.01 N hydrochloric acid. The data given in Table 5 show the comparative results of the oxidation of the various polyhydric alcohols when both yeast extract and alfalfa extract were used to supply nutrients. The data show that yeast extract and

TABLE 5

EFFECT OF DRY ALFALFA EXTRACT UPON THE CONVERSION OF POLYHYDRIC ALCOHOLS TO THE CORRESPONDING KETO-COMPOUNDS BY *A. suboxydans*

Substrate and Nutrient	Percentage Conversion of the Alcohol to the Ketose in		
	24 hrs.	48 hrs.	72 hrs.
5% Glycerol—0.5% yeast extract.....	75.3	94.2	93.8
“ “ “ alfalfa extract.....	44.6	76.1	91.0
5% Sorbitol—0.5% yeast extract.....	87.7	90.8	92.1
“ “ “ alfalfa extract.....	92.5	93.2	93.5
5% 2,3-butylene glycol—			
0.5% yeast extract.....	70.8	96.0	97.0
“ “ alfalfa extract.....	44.2	96.0	97.0

alfalfa extract have equal ability to bring about the desired conversions. In the fermentations of sorbitol and 2,3-butylene glycol the yields of keto-compounds were obtained equally as rapidly regardless of the nutrient employed. In the fermentation of glycerol the desired oxidation was completed in a shorter period of time with yeast extract, but the final yields were comparable. These results were confirmed by repeated experiments.

SUMMARY

1. Extracts of alfalfa have been prepared by heating alfalfa meal with water, with dilute hydrochloric acid or with dilute sodium hydroxide

under various conditions, and the ability of these extracts to supply the nutrients required for the growth of *Acetobacter suboxydans* has been investigated. The extracts supported the growth of the organism satisfactorily although not as well as yeast extract; best results were obtained with an acid extract.

2. A procedure has been described for the preparation of a dry material from the alfalfa extract. The growth of *Acetobacter suboxydans* using the best dry preparation to supply nutrients approached that obtained when an equal weight of yeast extract was employed.

3. The yields of keto-compounds from the polyhydric alcohols, glycerol, sorbitol, and 2,3-butylene glycol, were found to be the same when either yeast extract or alfalfa extract was used to supply nutrients.

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THE FERMENTABILITY OF THE STEREOISOMERIC 2,3-BUTANEDIOLS BY ACETOBACTER SUBOXYDANS¹

L. A. UNDERKOFER, E. I. FULMER, A. C. BANTZ, AND E. R. KOOI

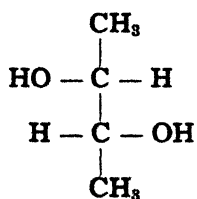
From the Department of Chemistry and the Industrial Science Research Institute, Iowa State College

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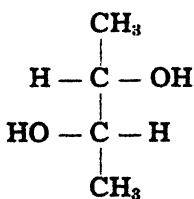
The organism *Acetobacter suboxydans* possesses a remarkable ability to oxidize polyhydric alcohols to the corresponding keto-compounds, and a considerable number of these oxidations have been studied quantitatively in these and other laboratories. The use of *A. suboxydans* frequently furnishes the most readily available method for the production of rare keto-compounds such as the ketose sugars.

Hann, Tilden, and Hudson (3) called attention to the fact that a specific relationship seemed to exist between the stereo-configuration of certain polyhydric alcohols and the ability of *A. suboxydans* to oxidize them. For instance, they found that *d*-arabitol was readily oxidized by the organism to *d*-xylulose whereas *l*-arabitol was not attacked. This is not surprising since it is well known that many living organisms are able to metabolize only one of a pair of enantiomorphs, and this "biochemical method" is sometimes employed for the resolution of racemic mixtures (2).

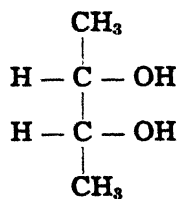
Only recently the stereoisomeric forms of 2,3-butanediol, commonly called 2,3-butylene glycol, have become available in sufficient quantities to permit study of their behavior in fermentations. Three stereoisomeric 2,3-butanediols are known, the configurations of which, according to conventional practice, may be represented as follows:



d-2,3-butanediol



l-2,3-butanediol



meso-2,3-butanediol

The present paper deals with the comparative ease of oxidation of these three stereoisomers to acetylmethylcarbinol by the action of *Acetobacter suboxydans*.

Fulmer, Underkofler, and Bantz (1) reported that better than 90 per cent of theory yields of acetylmethylcarbinol were obtainable by the action of *A. suboxydans* upon the 2,3-butylene glycol produced by the fermentation of dextrose by *Aerobacter aerogenes*. This glycol is now

¹This work was supported by a grant from the Industrial Science Research Institute of the Iowa State College for studies on the fermentative utilization of agricultural products.

known to be a mixture of the diastereoisomers, with the *meso*-glycol predominating. Fulmer, Underkofler, and Bantz concluded that their results indicated that *Acetobacter suboxydans* attacks the *meso*-glycol preferentially and that the glycol produced by the action of *Aerobacter aerogenes* consists of the *meso*-glycol and *dextro*-glycol with little or none of the *levo*-glycol. From the unfermented glycol residues from the fermentations they isolated 2,3-butylene glycol with the rotation $[\alpha]^{25}_D + 10.15$.

EXPERIMENTAL

The "aerobacter glycol" used in this investigation was obtained by the fermentation of dextrose by *Aerobacter aerogenes*. This product had a rotation of $[\alpha]^{25}_D + 1.0$, and from freezing point data (6) was found to contain about 90 per cent of the *meso*-2,3-butanediol. The *l*-2,3-butanediol was prepared in these laboratories by the fermentation of corn mashes by *Aerobacillus polymyxa*, according to the method discovered by workers at the Northern Regional Research Laboratory of the United States Department of Agriculture, and had a rotation of $[\alpha]^{25}_D - 13.0$. The *d*-2,3-butanediol was obtained from the residual unfermented glycol recovered from fermentations of the "aerobacter glycol" by *Acetobacter suboxydans*. This *dextro*-material had a rotation of $[\alpha]^{25}_D + 10.15$. If it be assumed that the *levo*-glycol with rotation of $[\alpha]^{25}_D - 13.0$ is the pure *l*-2,3-butanediol, then pure *d*-2,3-butanediol would have a rotation $[\alpha]^{35}_D + 13.0$. It is therefore apparent that the *dextro*-material employed, having a rotation $[\alpha]^{25}_D + 10.15$, predominated in *d*-2,3-butanediol but contained also some *levo*- or *meso*-glycol, probably the latter. The quantities of the *dextro*-material available were so meager that it was impractical to attempt to purify it further.

All fermentations were carried out in 50 ml. Erlenmeyer flasks containing 10 ml. of medium in each flask. In all cases the media were adjusted to pH 6.0 ± 0.1 and contained 5 per cent glycol, 0.5 per cent yeast extract, and 0.25 per cent maltose, the conditions shown in previous publications to be optimum (5, 1). The media were sterilized for 10 min. at 15 lbs. steam pressure.

The culture of *Acetobacter suboxydans* was originally obtained from the American Type Culture Collection listed as No. 621. Each experimental flask was inoculated with 6 drops of an active 24-hour culture of the organism growing on a 5 per cent glycol-0.5 per cent yeast extract-0.25 per cent maltose medium. Incubation was at 28°C.

The conversion of the glycol to acetylmethylcarbinol was followed by determining the content of reducing substance produced in the media, using a modified Shaffer-Somogi method developed in these laboratories (4).

All fermentations were run in duplicate, and the results were confirmed by repeated experiments. The data in Table 1 give the averages of the duplicate fermentations in a representative experiment, showing the yields of acetylmethylcarbinol obtained from the three glycol samples.

TABLE 1
EXTENT OF CONVERSION OF THE STEREOISOMERIC 2,3-BUTANEDIOLS TO ACETYLMETHYLCARBINOL
BY *Acetobacter suboxydans*

Substrate	Percentage Conversion of the Glycol in		
	24 hrs.	48 hrs.	96 hrs.
<i>l</i> -2,3-butanediol	35	88	96
" <i>Aerobacter glycol</i> " (90% <i>meso</i> -2,3-butanediol)	27	72	95
<i>d</i> -2,3-butanediol (predominately)	0.9	1.3	3.3

The results show clearly that *A. suboxydans* preferentially oxidizes the *meso*- and *levo*-compounds. The *levo*-glycol is attacked somewhat more rapidly than the *meso*-compound. The *dextro*-glycol is acted upon by the organism only very slightly, if at all; the small reducing values obtained in the fermentations of the *dextro*-glycol were probably due to the presence of *meso*-glycol in this material. It is evident that *A. suboxydans* is able to oxidize only the *meso*- 2,3-butanediol and the *levo*-rotatory enantiomorph of the active 2,3-butanediols.

SUMMARY

It has been demonstrated that *Acetobacter suboxydans* oxidizes *meso*-2,3-butanediol and *l*-2,3-butanediol to acetylmethylcarbinol, but does not attack the *d*-2,3-butanediol. This represents another example of the biochemical specificity of living organisms toward one of a pair of enantiomorphs.

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FLORA OF ALASKA AND ADJACENT PARTS OF CANADA
AN ILLUSTRATED DESCRIPTIVE TEXT OF ALL VASCULAR PLANTS KNOWN
TO OCCUR WITHIN THE REGION COVERED

PART II. TYPHACEAE TO POACEAE

J. P. ANDERSON

From the Department of Botany, Iowa State College

Received February 12, 1944

Class 2. ANGIOSPERMAE

- Cotyledons 1, leaves mostly parallel-veined, stems endogenous.....
.....Subclass 1. *Monocotyledoneae*
Cotyledons 2, leaves mostly net-veined, stems exogenous.....
.....Subclass 2. *Dicotyledoneae*

Subclass 1. MONOCOTYLEDONEAE

- 1A. Very small, free-floating plants without differentiation into stem and
leavesFamily 9. *Lemnaceae*
2A. Plants rooted in the soil.
1B. Strictly marine plants with ribbon-like leaves.....
.....Family 4. *Zosteraceae*
2B. Not marine but often growing in brackish water.
1C. Plants submerged, but often with floating leaves.....
.....Family 3. *Potamogetonaceae*
2C. Plants terrestrial or if aquatic, emersed.
1D. Flowers monoecious, aquatic or marsh plants.
1E. Flowers in an elongated terminal spike.....
.....Family 1. *Typhaceae*
2E. Flowers in dense spherical heads
.....Family 2. *Sparganiaceae*
3E. Flowers on a fleshy axis (spadix) and subtended by a
large, conspicuous, fleshy bract (spathe).....
.....Family 8. *Araceae*
2D. Flowers perfect.
1E. Perianth conspicuous.
1F. Ovary superior.
1G. Styles distinctFamily 11. *Melanthaceae*
2G. Styles united.
1H. Herbs with bulbs
.....Family 12. *Liliaceae*
2H. Herbs with rootstocks
.....Family 13. *Convallariaceae*

- 2F. Ovary inferior.
 - 1G. Perianth radial (regular) Family 14. *Iridaceae*
 - 2G. Perianth bilateral (irregular) Family 15. *Orchidaceae*
- 2E. Perianth inconspicuous and scaly or absent. (See also *Tofieldia* in *Melanthaceae*.)
- 1F. Perianth 6-parted.
 - 1G. Perianth fleshy, inflorescence a raceme, usually spike-like Family 5. *Scheuchzeriaceae*
 - 2G. Perianth scalelike, inflorescence umbellate or paniculate Family 10. *Juncaceae*
- 2F. Perianth absent or represented by 1 or 2 minute scales. Grasslike plants.
 - 1G. Leaves 2-ranked, fruit a grain..... Family 6. *Poaceae*
 - 2G. Leaves 3-ranked, fruit an achene Family 7. *Cyperaceae*

1. TYPHACEAE (Cat-tail Family)

Aquatic or marsh plants; leaves long, linear, flat, striate, sheathing at the base; flowers monoeious, in dense terminal spikes with the staminate part uppermost; perianth of bristles; stamens 2-7; ovary stipitate, 1-2-celled.

TYPHA (Tourn.) L.

The only genus. (Name ancient.)

T. latifolia L.

Common Cat-tail

Stem stout, 1-2.5 m. tall; leaves 6-25 mm. wide; spikes dark brown, the staminate portion lighter than the pistillate, and with bractlets; the pistillate portion without bractlets.

In ponds near Fairbanks. Circumboreal. (Fig. 60.)

2. SPARGANIACEAE (Bur-reed Family)

Marsh or water plants with creeping rootstocks; leaves linear, alternate, clasping at the base; flowers monoecious, in dense, globose heads, the staminate uppermost, the pistillate below, the lower ones peduncled; perianth reduced to a few scales; fruit nutlike.

SPARGANIUM (Tourn.) L.

The only genus (Greek, referring to the ribbon-like leaves).

- 1A. Peduncles of the upper pistillate heads adnate to the stem (super-axillary).
 - 1B. Beak very short or lacking.....1. *S. hyperboreum*
 - 2B. Beak nearly as long as the achenes.
 - 1C. Staminate heads remote2. *S. simplex*
 - 2C. Staminate heads approximate3. *S. angustifolium*
- 2A. Pistillate heads all strictly axillary.....4. *S. minimum*

1. *S. hyperboreum* Laest.

Northern Bur-reed

Stem floating and elongated or when growing in mud decumbent and ascending, 1-2 dm. tall; leaves linear, 6-40 cm. long, 1-4 mm. wide, the sheaths somewhat dilated near the base; staminate heads 1 or 2, close to the upper pistillate ones; pistillate heads 2-4, the upper sessile, the lower peduncled, in fruit 8-11 mm. in diameter; achenes ellipsoid.

Generally distributed in our territory except the extreme arctic. Circumboreal. (Fig. 61.)

2. *S. simplex* Huds.

Simple-stemmed Bur-reed

Stem rather stout, 4-6 dm. tall, leaves linear, keeled, 4-9 dm. long, 8-15 mm. wide; inflorescence simple; staminate heads 4-8, pistillate heads 2-5, about 15 mm. in diameter at maturity; achenes stipitate, fusiform, 5-6 mm. long, often constricted at the middle; stigma linear.

Extreme southern part of southeastern Alaska. Circumboreal.

3. *S. angustifolium* Michx.

Narrow-leaved Bur-reed

S. affine Schniz.

S. multipedunculatum (Morong) Rydb.

Stems floating and elongated or erect and 2-5 dm. tall; leaves 2-6 dm. long, 3-8 mm. wide, dilated and scarious-margined at base, more or less reticulated; staminate heads approximate but distant from the pistillate ones; pistillate heads 2-4, the lower ones peduncled, in fruit 15-20 mm. in diameter; achenes stipitate, fusiform, brown; stigma about 1 mm. long.

Aleutians and Bering Strait—Greenl.—Penn.—Calif. Also N. Europe and Kamtchatka. (Fig. 62.)

4. *S. minimum* (Hartm.) Fr.

Small Bur-reed

Stem usually slender and floating, 1-4 dm. long; leaves flat, thin, 2-6 mm. wide, bases of upper ones dilated; pistillate heads 1-3, axillary, the lower sometimes peduncled, less than 1 cm. wide in fruit; achenes ellipsoid to obovoid, sometimes constricted below the middle, short-beaked.

Central Alaska south and east. Circumboreal. (Fig. 63.)

3. POTAMOGETONACEAE (Pondweed Family)

Perennial, mostly fresh-water plants with slender branching stems and floating or submerged leaves or both. Flowers perfect or monoecious, in axillary spikes or clusters; perianth none but flowers sometimes enclosed in hyaline envelopes; stamens 1-4; pistil of 1-4 distinct, 1-celled, 1-ovuled carpels; fruit small drupelets.

1A. Flowers perfect.

1B. Stamens 4, drupelets sessile1. *Potamogeton*

2B. Stamens 2, drupelets stipitate2. *Ruppia*

2A. Flowers monoecious, stamen 13. *Zannichellia*

1. POTAMOGETON (Tourn.) L.

Leaves alternate or the upper opposite, often of 2 kinds, the submerged thin, pellucid and narrow, the floating broader and coriaceous; stipules present, enclosing the young flower buds; inflorescence spicate, axillary, usually emersed; stamens 4, the connective tissue sometimes becoming perianth-like; carpels 4, distinct; fruit of 4 drupelets. (Greek, in allusion to the aquatic habit.)

1A. Leaves of 2 sorts, floating and submerged.

1B. Submerged leaves without proper blades.....1. *P. natans*

2B. Submerged leaves 2 mm. or more wide.

1C. Submerged leaves very finely denticulate.....
.....2. *P. gramineus*

2C. Submerged leaves all entire.

1D. Submerged leaves ribbon-like3. *P. epihydrus*2D. Submerged leaves narrowly lanceolate
.....4. *P. alpinus*

2A. Leaves all submerged.

1B. Stipules free, spike compact.

1C. Stem flattened, leaves narrow.

1D. Leaves 9-17-nerved5. *P. porsildorum*2D. Leaves 1-3-nerved6. *P. pusillus*3D. Leaves 5-nerved7. *P. friesii*

2C. Stem not conspicuously flattened, leaves broader.

1D. Leaves with broad blades.

1E. Leaves half-clasping8. *P. praelongus*2E. Leaves cordate-clasping9. *P. perfoliatus*2D. Leaves narrowly lanceolate2. *P. gramineus*

2B. Stipules adnate, spike interrupted.

1C. Stigmas sessile.

1D. Leaves filiform10. *P. filiformis*2D. Leaves narrowly linear11. *P. interior*

2C. Stigmas on a distinct style.

1D. Leaves blunt12. *P. vaginatus*2D. Leaves acute13. *P. pectinatus*1. *P. natans* L.

Floating Pondweed

Stems simple or sparingly branched, 6-14 dm. long; floating leaves ovate or elliptical, thick, short-pointed at the apex, rounded or cordate at the base, 4-10 cm. long, 2-5 cm. wide, on rather long petioles; submerged leaves of bladeless petioles and early perishing; stipules 5-10 cm. long, acute, 2-keeled; spike cylindric, dense, 3-5 cm. long; drupelets obovoid, 4-5 mm. long; stone 2-grooved on the back.

Coast districts, not common. Circumboreal and nearly cosmopolitan. (Fig. 64.)

2. *P. gramineus* L.

Various-leaved Pondweed

P. heterophyllus Schreb.

Stems slender, branching, and often very long; floating leaves usually present, oval, pointed at the apex, usually rounded at the base, 1.5-8 cm.

long, 8–28 mm. wide, 9–19-nerved; submerged leaves linear or linear-lanceolate, pellucid, reticulated, 5–15 cm. long, 2–16 mm. wide, 3–9-nerved; peduncles 3–8 cm. long; spikes 2–4 cm. long, many flowered; drupelets indistinctly 3-keeled, 2–3 mm. long.

From Bering Str. east and south. Circumboreal. (Fig. 65.)

3. *P. epihydrus* Raf.

Nuttall Pondweed

P. epihydrus Raf. var. *nuttallii* (Cham. & Schlecht.) Fern.

Stems slender, compressed, 3–18 dm. long; floating leaves opposite, elliptic to obovate, petioled, obtuse, narrowed at the base, 3–8 cm. long, 6–18 mm. wide, many nerved; submerged leaf-blades linear or linear-lanceolate, 2–4 mm. wide, reticulate along the midrib, 5-nerved, the outer nerves nearly marginal; spikes cylindric, many-flowered, 1.5–6 cm. long; drupelets round-ovoid, pitted, 3-keeled; style short, apical.

Revillagigedo Island—Labr.—Newf.—Ga.—Colo.—Calif.

4. *P. alpinus* Balbis var. *tenuifolius* (Raf.) Fern. Northern Pondweed

Plants with a ruddy tinge, simple or branching; floating leaves oblanceolate or spatulate, 5–12 cm. long, sometimes wanting; submerged leaves thin, oblong to linear-lanceolate, 7–30 cm. long; spikes cylindric, 2–4 cm. long; drupelets ovoid, lenticular, 2.5–3.5 mm. long, with a sharp middle keel and a short recurved style.

Most of our territory south of the Arctic Circle. Circumboreal. The var. *tenuifolius* is eastern Asiatic and American. (Fig. 66.)

5. *P. porsildorum* Fern.

Porsild Pondweed

Stem simple or branching, 1–6 dm. long; leaves 3.5–9.5 cm. long, 1.5–2 mm. wide, 9–17-nerved, apex rounded, subacute to mucronate; base with 2 prominent glands; stipules subrigid, subpersistent, many-nerved, 1–2.5 cm. long; spikes with 3 or 4 verticils; drupelets oblong-ovoid, 3–4 mm. long, 1.5–2 mm. wide, base obliquely truncate.

Known from Buckland River and Takotna to the Mackenzie Delta and James Bay. (Fig. 67.)

6. *P. pusillus* L.

Small Pondweed

Entirely submerged with filiform branched stem; leaves linear, 2–6 cm. long, with strong midrib and usually inconspicuous side veins, 1–1.5 mm. wide, inconspicuous glands at base, the tip often narrowed into a short acumination; peduncles 6–25 mm. long; spikes few-flowered, about 5 mm. long; drupelets broadly ovoid, about 2 mm. long, indistinctly 3-keeled. A variable species, of which three varieties of doubtful distinction have been reported from Alaska.

Bering Sea region south and east. Circumboreal. (Fig. 68.)

7. *P. friesii* Rupr.

Fries Pondweed

Stems branching, 4–12 dm. long; leaves linear, 3–6 cm. long, about 2 mm. wide, usually 3-nerved, acute or cuspidate at apex, 2-glandular at base; stipules white, finely nerved, 10–20 mm. long; peduncles often

thicker than the stem; mature spikes often somewhat interrupted; druplets with recurved style and usually a shallow pit on sides.

Matanuska. Circumboreal. (Fig. 69.)

8. *P. praelongus* Wulf.

White-stemmed Pondweed

Stems white, flexuous, much-branched, somewhat flattened, up to 25 dm. long; leaves oblong-lanceolate, 5-25 cm. long, 15-30 mm. wide, with 3-5 main nerves; stipules white, scarious, 15-30 mm. long; spikes cylindric, thick, 2-4 cm. long; druplets slightly keeled, 4-5 mm. long.

Atka and Kodiak. Circumboreal.

9. *P. perfoliatus* L.

Clasping-leaved Pondweed

P. perfoliatus L. var. *gracilis* Fries.

P. richardsonii (A. Benn.) Rydb.

Stems very leafy; leaves all submerged, thin, lanceolate, with cordate-clasping base, 4-10 cm. long, 8-15 mm. wide; stipules usually conspicuous, often in shreds; peduncles 3-10 cm. long, thickened upward and somewhat spongy; spikes cylindric, 2-3.5 cm. long; druplets obscurely 3-keeled, 3-4 mm. long. This species is represented in central and western Alaska by a near typical form (var. *gracilis* Fries) and in the coastal districts by var. *richardsonii* A. Benn. which has narrower leaves and longer stipules.

Circumboreal. Also northern Africa and southern Australia. (Fig. 70.)

10. *P. filiformis* Pers.

Filiform Pondweed

Stems from running rootstocks, branching, slender above, stouter toward the base; leaves linear-filiform, 5-30 cm. long, less than 1 mm. wide; sheaths 2-3 cm. long; peduncles 4-7 cm. long; spikes interrupted, the verticels 3-20 mm. apart; druplets ovoid, 2-3 mm. long, nearly 2 mm. wide; stigma sessile, forming a broad truncate projection on the druplet.

In most parts of Alaska and Yukon. Circumboreal. (Fig. 71).

11. *P. interior* Rydb.

Interior Pondweed

Stems slender, much-branched; leaves capillary or linear, 3-15 cm. long, about 1 mm. wide, mostly 1-nerved, with acute, pungent apex; adnate portion of stipules 14 mm. or more long, free portion 2-4 mm. long; spikes few-flowered, 15-85 mm. long; druplets obliquely ovoid, 2-grooved on back; stigma subsessile.

Pacific coast districts—Ont.—N. Mex.—Calif.

12. *P. vaginatus* Turcz.

Sheathed Pondweed

Stem compressed, 4-12 dm. long; leaves 0.5-2 mm. wide, up to 3 dm. long; peduncles filiform, 5-10 cm. long; spikes 3-5 cm. long, interrupted; druplets 2.5-3 mm. long, without keel. Related to *P. pectinatus*.

In brackish and salt water, Bering Sea and Pacific coasts. More or less circumboreal.

13. *P. pectinatus* L.

Fennel-leaved Pondweed

Stems filiform, much-branched, the branches repeatedly forking; leaves narrowly linear or setaceous, attenuate at the apex, 3–15 cm. long, less than 1 mm. wide; stipular sheaths 1–2 cm. long, adnate one-half their length or more; peduncles filiform, 5–25 cm. long; spikes interrupted with 2–6 verticels; drupelets obliquely ovoid, 3–4 mm. long, rounded on the back and with 2 obscure keels.

Matanuska, Cordova, and Circle. Cosmopolitan. (Fig. 72.)

2. RUPPIA L.

Slender, widely-branched water plants with capillary stems and filiform, alternate leaves with membranous sheaths at the base. Flowers on a capillary spadix-like peduncle which becomes long and coiled in fruit; flowers consisting of 2 sessile anthers and 4 pistils, sessile at first, in fruit long-stipitate; fruit a small obliquely-pointed drupelet. (Heinrich Bernhard Rupp was a German botanist.)

Stipular sheaths 15 mm. long.....1. *R. spiralis*
Stipular sheaths 20 mm. long2. *R. canadensis*

1. *R. spiralis* L.

Ditch-grass. Widgeon-grass

Stems much branched, often long; leaves up to 15 cm. long, less than 0.5 mm. wide, and with a sharp tip; stipular sheaths 6–15 mm. long; peduncles in fruit elongating and coiling into a loose spiral; fruit ovoid, about 2 mm. long, obliquely attached.

Salt and brackish water along the coast from St. Paul Island eastward. Cosmopolitan in distribution. (Fig. 73.)

2. *R. canadensis* S. Wats.

Western Ditch-grass

R. lacustris Macoun.

Differs from *R. spiralis* in having stipules 2–4 cm. long, leaves up to 25 cm. long, and generally stouter stems.

Unalaska Island and B. C.—Wash.—Nebr.

3. ZANNICHELLIA L.

Submerged aquatics with capillary, sparsely-branched stems; leaves linear-filiform, 1-nerved; staminate and pistillate flowers in the same axil, the staminate of a single 2-celled anther on a short pedicel-like filament, the pistillate of 2–6 sessile pistils in a cup-shaped involucre; fruit a flattish falcate nutlet with a slender beak, ribbed or toothed on the back. (J. H. Zannichelli was an Italian physician and botanist.)

Z. palustris L.

Horned Pondweed

Stems capillary from creeping rhizomes; leaves 2–10 cm. long, 0.5 mm. or less wide, acute at the apex; fruits 2–6 together, 2–4 mm. long, sometimes pedicelled.

In Alaska, known only from the delta of the Buckland River. Cosmopolitan in distribution.

4. ZOSTERACEAE (Eel-grass Family)

Submerged marine plants with creeping rootstocks, flattened branching stems, 2-ranked, ribbon-like leaves, monoecious or dioecious flowers borne on a spadix, enclosed in a spathe, and without perianth but enclosed in a hyaline scale. Staminate flowers consisting of single 1-celled anthers in 2 rows on the spadix which produce filamentous pollen; pistillate flowers of single, 1-celled ovaries composed of two carpels.

Flowers monoecious1. *Zostera*

Flowers dioecious2. *Phyllospadix*

1. ZOSTERA L.

Marine plants with 2-ranked leaves sheathing at the base, the sheaths with inflexed margins; flowers arranged alternately in two rows on the spadix; pollen threadlike; pistillate flowers fixed on the back near the middle; style elongated; stigma capillary; mature carpels flask-shaped, beaked. (Greek, referring to the ribbon-like leaves.)

Z. marina L.

Eel-grass

Stems branched, arising from a thickish rootstock; leaves ribbon-like, obtuse at apex, 3-15 dm. long, 2-8 mm. wide; spadix 25-60 cm. long, the flowers crowded; at anthesis the anthers escaping and releasing the glutinous, filamentous pollen in the water; fruit strongly 20-ribbed, about 3 mm. long and 1 mm. wide.

Along the coast from Bering Strait south. Circumboreal, but absent from most of the Arctic coasts. (Fig. 74.)

2. PHYLLOSPADIX Hook.

Rootstocks thickened, stems slender, bearing the inflorescence at the summit; leaves linear, sheathing; flowers in spathes, the spadix with a series of short, dilated, foliaceous flaps, which close over the flower; staminate flowers of numerous sessile anthers in 2 rows, producing threadlike pollen; pistillate flowers of single sessile ovaries, tapering into a short style with 2 stigmas; fruit beaked, cordate-sagittate. (Greek, referring to the leaflike appendages of the spathe.)

P. scouleri Hook.

Scouler Surf-grass

Stem winged, 1-4 dm. long; leaves 2-4 mm. wide with 3 primary nerves.

On rocks in the surf, Sitka. Coasts of Japan and B. C.—Calif.

5. SCHEUCHZERIAACEAE (Arrow-grass Family)

Marsh herbs with rushlike leaves and small, perfect flowers in spikes or panicles. Perianth 4-6-parted in 2 series; stamens 3-6; anthers 2-celled; carpels 3-6, 1-2-ovuled, more or less united but separating at maturity.

Stems scapose1. *Triglochin*

Stems leafy2. *Scheuchzeria*

1. TRIGLOCHIN L.

Seaside or marsh herbs with half-round, elongated, linear leaves, sheathing at the base; flowers in long terminal racemes or spikes on a naked scape; stamens 6, the anthers sessile or nearly so; carpels 3-6, 1-celled, 1-ovuled, united at first, at maturity separating from the base upward; stigmas plumose; seed compressed or angular. (Greek, referring to the fruit of the 3-carpelled species.)

Carpels 6, fruit obtuse at base1. *T. maritima*
 Carpels 3, fruit with subulate base2. *T. palustris*

1. *T. maritima* L.

Seaside Arrow-grass

Scape stout, 1-10 dm. tall; leaves 1-6 dm. long, about 3 mm. wide; raceme often 4 dm. long; pedicels 2-4 mm. long, decurrent along the stem, ascending in fruit; fruit 5-6 mm. long, 3-5 mm. wide; carpels triangular, depressed on the back. A form collected near mile 280 on Richardson Highway has fruit only 3-4 mm. long.

Beaches and salt meadows, Kotzebue southward. Occasional in interior from Wiseman south. Circumboreal and to S. America. (Fig. 75.)

2. *T. palustris* L.

Marsh Arrow-grass

Scape slender, 1-5 dm. tall; leaves slender, tapering to a sharp point, 5-30 cm. long; pedicels slender, 2-6 mm. long, erect in fruit; fruit 6-7 mm. long, about 1.5 mm thick, pointed at the lower end.

On very wet soil, Kotzebue and Wiseman south. Circumboreal and in Chile. (Fig. 76.)

2. SCHEUCHZERIA L.

Rushlike, bog plants with creeping rhizomes and erect, leafy stems; leaves elongated, striate, half-round below, flat above, with pore at the apex; flowers small, regular, perfect; perianth 6-parted in 2 series, persistent; stamens 6; anthers linear; ovaries 3, rarely more, separate or connected at the base; stigmas sessile, carpels divergent, 1- or 2-seeded. (Johan Jacob Scheuchzer was a Swiss scientist.)

S. palustris L.

Scheuchzeria

Stems leafy, 1-3 dm. tall; leaves 1-4 dm. long, the upper reduced to bracts; sheaths of the lower leaves up to 1 dm. long; flowers white, segments 1-nerved, 3 mm. long; pedicels 6-20 mm. long, in fruit spreading; follicles 5-9 mm. long.

Extreme southern part of southeastern Alaska. Circumboreal. The American form has longer follicles and styles than the European and has been described as var. *americana* Fern.

6. POACEAE (Grass Family)

Herbs, or in warm climates sometimes woody plants with usually hollow stems (culms) closed at the joints and 2-ranked, parallel-veined

leaves, the lower portion forming a sheath enveloping the culm, with an appendage (ligule) at the junction of sheath and blade. Flowers usually perfect, small, with no typical perianth, arranged in spikelets consisting of a shortened axis (rachilla) and 2 to many 2-ranked bracts, the lowest 2 (glumes) empty or rarely obsolete, the succeeding 1 or more (lemmas) bearing a single floret in the axil, and between the floret and the rachilla a second 2-nerved bract (palea); stamens usually 3; pistil of a 1-celled, 1-ovuled ovary with usually 2 styles; fruit a seedlike grain (caryopsis). A large family of cosmopolitan distribution and the most valuable of all plants. Here belong corn, wheat, oats, rye, barley, rice, sugar-cane, bamboo, the sorghums, millet, and most of the hay and forage crops. Indirectly it furnishes most of our meats by furnishing the bulk of the food for all grazing animals.

The family is divided into 2 subfamilies, *Festucoidae* and *Panicoidae*. The latter is not represented in our area. The subfamilies are divided into tribes of which six are represented in our area.

- 1A. Spikelets distinctly pedicelled, panicles sometimes contracted and spike-like.
 - 1B. First (lowest) and second florets staminate or neuter.....1. *Phalarideae*
 - 2B. Lowest floret perfect, imperfect florets, if any, uppermost.
 - 1C. Spikelets 1-flowered2. *Agrostideae*
 - 2C. Spikelets 2-many-flowered.
 - 1D. Lemmas awned on back, glumes longer than the lemmas....3. *Aveneae*
 - 2D. Lemmas awnless or with terminal awn, glumes usually shorter than the lemmas5. *Festuceae*
- 2A. Spikelets sessile in spikes.
 - 1B. Spikes unilateral4. *Chlorideae*
 - 2B. Spikes not unilateral6. *Hordeae*

1. Phalarideae

- 1A. Lower floret staminate; spikelets brown and shining.....1. *Hierochloe*
- 2A. Lower floret neuter, spikelet green or yellowish.
 - 1B. Lower florets reduced to small awnless scalelike lemmas; spikelets compressed laterally2. *Phalaris*
 - 2B. Lower florets consisting of awned, hairy lemmas; spikelets subterete3. *Anthoxanthum*

2. Agrostideae

- 1A. Lemmas with long terminal awn and closely enveloping the grain.....4. *Stipa*
- 2A. Lemmas awnless or short-awned; awn when present dorsal.
 - 1B. Entire spikelet deciduous at maturity.
 - 1C. Glumes awnless5. *Alopecurus*
 - 2C. Glumes awned6. *Polypogon*

2B. Lemmas deciduous above the glumes.

1C. Glumes awned 7. *Phleum*

2C. Glumes awnless.

1D. Lemmas 1-nerved 8. *Phippsia*

2D. Lemmas 3-5-nerved.

1E. Stamen 1; lemma stipitate 9. *Cinna*

2E. Stamens 3, lemmas sessile.

1F. Lemmas copiously hairy at base....10. *Calamagrostis*

2F. Lemmas naked or short-hairy at base.

1G. Glumes longer than the lemmas, spikelets small....

.....11. *Agrostis*

2G. Glumes shorter than the lemmas, spikelets large....

.....12. *Arctagrostis*3. *Aveneae*

1A. Spikelets with 1 perfect and 1 staminate floret.

1B. Lower floret perfect, awnless, upper staminate with hooked awn....

.....13. *Holcus*

2B. Lower floret staminate, awned, upper perfect, awnless.....

.....14. *Arrhenatherum*

2A. Perfect florets 2 or more.

1B. Lemmas usually awnless.

1C. Articulation below the glumes, glumes dissimilar.....

.....15. *Sphenopholis*

2C. Articulation above the glumes, glumes nearly alike.....

.....16. *Koeleria*

2B. Lemmas with twisted awn arising from between 2 terminal teeth

.....17. *Danthonia*

3B. Lemmas with dorsal awn.

1C. Spikelets large, more than 1 cm. long.....18. *Avena*

2C. Spikelets small, less than 1 cm. long.

1D. Lemmas keeled, awn arising from above the middle

.....19. *Trisetum*

2D. Lemmas convex, awn arising from below the middle.....

.....20. *Deschampsia*4. *Chlorideae*Glumes equal, broad boat-shaped21. *Beckmannia*5. *Festuceae*1A. Spikelets nearly sessile in dense one-sided clusters at the ends of the
few panicle branches22. *Dactylis*

2A. Spikelets not as above.

1B. Callus barbellate or pilose.

1C. Panicle erect, the rigid branches often divergent.....

.....23. *Dupontia*

2C. Panicle nodding, the spreading branches capillary.

1D. Lemmas awned 24. *Schizachne*2D. Lemmas awnless25. *Colpodium*

2B. Callus naked.

1C. Lemmas rounded on back.

1D. Nerves of the lemmas prominent.

1E. Lemmas long acuminate-pointed.....26. *Melica*2E. Lemmas obtuse27. *Glyceria*

2D. Nerves of lemmas obscure or evident only near the apex.

1E. Lemmas obtuse, awnless.

1F. Glumes usually small and shorter than the lemmas....
.....28. *Puccinellia*2F. Glumes usually about as long as the nearest lemma,
the lemma usually more or less pubescent.....
.....29. *Poa*

2E. Lemmas acute or obtuse, often awned.

1F. Lemmas acute or awned from the apex.....
.....30. *Festuca*2F. Lemmas obtuse, usually awned from below the apex
.....31. *Bromus*

2C. Lemmas compressed-keeled.

1D. Spikelets 1 cm. or more long.....31. *Bromus*2D. Spikelets less than 1 cm. long.....29. *Poa*6. **Hordeae**

1A. Spikelets solitary at each node of the rachis.

1B. Spikelets placed edgewise to the rachis.....32. *Lolium*2B. Spikelets placed flatwise to the rachis.....33. *Agropyron*

2A. Spikelets 2 or 3 at each node of the rachis.

1B. Spikelets 1-flowered34. *Hordeum*2B. Spikelets several-flowered35. *Elymus*1. **HIEROCHLOE** R. Br.*Savastana* Schrank.*Torresia* Ruiz & Pav.

Perennial, erect, sweet-smelling grasses with small panicles of broad, bronze-colored spikelets; spikelets 3-flowered, the terminal floret perfect, the others staminate; glumes equal, 3-nerved, broad, smooth, acute; staminate lemmas about as long as the glumes, boat-shaped, hispidulous, hairy along the margins; fertile lemma indurate; smooth or nearly so, awnless. (Greek, sacred plus grass.)

1A. Staminate lemmas awned1. *H. alpina*

2A. Staminate lemmas awnless.

1B. Culm 16 cm. tall or less2. *H. pauciflora*2B. Culm 20 cm. long or more3. *H. odorata*1. *H. alpina* (Sw.) Roem. & Schult.

Alpine Holy-grass

Savastana alpina (Sw.) Scribn.

Culms tufted, 1-4 dm. tall, with leafy shoots at the base and short rhizomes; blades 1-2 mm. wide, those of the culm short and wider; panicle

2–4 cm. long; spikelets 5–8 mm. long; glumes glabrous; staminate lemmas ciliate on the margins, the first with a short straight awn, the second with a bent awn, 5–8 mm. long; fertile lemma pubescent near the apex.

Alpine-arctic situations throughout our territory. Circumboreal. (Fig. 77.)

2. *H. pauciflora* R. Br.

Arctic Holy-grass

Savastana pauciflora (R.Br.) Scribn.

Stems glabrous, erect, simple; basal sheaths overlapping; blades about 1 mm. wide, up to 7 cm. long, involute when dry; stem leaves flat, short and wider, the uppermost almost obsolete; panicle 1–2.5 cm. long, contracted; spikelets few, 3–5 mm. long; glumes smooth and glabrous; staminate lemmas scabrous, erose-truncate; fertile lemma shorter than the others, obtuse with villous apex.

Arctic regions. Circumpolar. (Fig. 78.)

3. *H. odorata* (L.) Beauv.

Holy-grass. Sweet-grass

Savastana odorata (L.) Scribn.

Torresia odorata (L.) Hitchc.

Culms 2–6 dm. tall with some leafy shoots and creeping rhizomes; blades 2–6 mm. wide, those of the sterile shoots elongate; panicle 4–12 cm. long, open; spikelets about 5 mm. long; lemmas awnless or nearly so, brown-pubescent.

From about the Arctic Circle south. Circumboreal. (Fig. 79.)

2. PHALARIS L.

Grasses with numerous flat leaves and narrow or spike-like inflorescence; spikelets crowded, laterally compressed, with 1 terminal perfect floret and 2 sterile lemmas below, the rachilla disarticulating above the glumes, the usually inconspicuous sterile lemmas falling close appressed to the fertile floret; glumes equal, boat-shaped, often winged on the keel; fertile lemma coriaceous, enclosing the faintly 2-nerved palea. (Greek, alluding to the shining grain.)

P. arundinacea L.

Reed Canary-grass

Perennial with creeping rhizomes; culms 6–22 dm. tall, glaucous; leaves 6–18 mm. wide, up to 3 dm. long; panicle 7–18 cm. long, the branches spreading during anthesis, later erect; glumes about 5 mm. long, 3-nerved, acute, the keel scabrous; fertile lemma lanceolate, 4 mm. long, with a few appressed hairs; sterile lemmas villous, about 1 mm. long.

Wet places, central Alaska south and east—N. B.—N. Car.—Okla.—Ariz.—Calif. (Fig. 80.)

P. canariensis L., Canary-grass, which furnishes the chief constituent of the bird seed of commerce has been collected a few times, but it is not yet known to be able to maintain itself. It is a glabrous annual with stems branched at the base, 3–9 dm. tall; leaves 4–12 mm. wide; the spikelets broad, imbricate, in a dense, ovoid, headlike panicle; glumes pale with

green stripes and a narrowly winged keel. Native of the Mediterranean region.

3. ANTHOXANTHUM L.

Fragrant grasses with spike-like panicle inflorescence; spikelets with 1 terminal perfect floret, 2 sterile lemmas and unequal glumes; glumes acute or acuminate; sterile lemmas awned from the back, shorter than the glumes but longer than the fertile lemma; fertile lemma awnless; stigmas elongated, plumose. (Greek, referring to the yellowish color of the spikelets of some species.)

A. odoratum L.

Sweet Vernal-grass

A tufted perennial 3–6 dm. tall; sheaths shorter than the internodes; leaves 2–5 mm. wide, flat; spike-like panicle greenish yellow-brown, 2–6 cm. long; spikelets 8–10 mm. long; first glume 1-nerved, half as long as the 3-nerved second glume; sterile lemmas subequal, appressed-pilose, the first with a straight awn from near the middle, the second with a long geniculate awn from near the base; fertile lemma smooth and shining, about 2 mm. long.

Established at Unalaska and perhaps elsewhere in Alaska. Native of Eurasia. (Fig 81.)

4. STIPA L.

Tufted perennial grasses; leaves usually convolute; panicles mostly narrow; spikelets 1-flowered, disarticulating above the glumes, the articulation oblique, leaving a bearded, sharp-pointed callus attached to the base of the floret; glumes membranous, acute, acuminate, or aristate; lemma narrow, terete, firm, enclosing the palea and jointed to a usually bent and twisted awn. (Greek, in reference to the feathery awns of the type species.)

Awn 2–2.5 cm. long1. *S. columbiana*

Awn 10–15 cm. long2. *S. comata*

1. *S. columbiana* Macoun.

Columbia Needle-grass

Stems 3–6 dm. or more tall; sheaths smooth; ligule short; blades 1–3 dm. long, 1–3 mm. wide, mostly involute, those of the stem sometimes flat; panicle 6–20 cm. long, narrow, rather dense, often purplish; glumes about 1 cm. long; lemmas 6–7 mm. long, pubescent; awn twice geniculate, 2–3 cm. long.

Dry plains and meadows, Yukon—Texas—Calif. [Fig. 82 (from a Wyo. specimen).]

2. *S. comata* Trin.

Needle and Thread

Stems 3–6 dm. or more tall; sheaths usually longer than the internodes, smooth or scabrous, the upper one long and inflated, enclosing the base of the panicle; ligule 3–4 mm. long; basal leaves involute-filiform, those of the stem somewhat wider; panicle 1–2 dm. long; glumes 15–20

mm. long with attenuated tips; lemmas 8-12 mm. long, finally brownish with callus about 3 mm. long; awn 10-15 cm. long, very slender, flexuous, indistinctly twice geniculate.

Plains, prairies, and dry hills, Yukon—Ind.—Texas—Calif. [Fig. 83 (from a Wyo. specimen).]

5. ALOPECURUS L.

Ours perennial grasses with flowers in dense spike-like panicles; spikelets 1-flowered, disarticulating below the glumes, compressed; glumes equal, united at the base, ciliate on the keel; lemmas about as long as the glumes, 5-nerved, obtuse, with dorsal awn or point, the margins united at the base; palea none. (Greek, fox plus tail.)

1A. Spikelets 5-6 mm. long1. *A. pratensis*

2A. Spikelets 2-4 mm. long.

1B. Spikelets densely wooly all over.

1C. Tall-growing, up to 1 m.2. *A. glaucus*

2C. Lower-growing, 15-50 cm.3. *A. alpinus*

2B. Spikelets not densely wooly all over.

1C. Awn scarcely exceeding the glumes.....4. *A. aequalis*

2C. Awn exerted 2-3 mm.5. *A. geniculatus*

1. *A. pratensis* L.

Meadow Foxtail

Culms erect, 3-10 dm. tall; leaves 2-6 mm. wide; panicle 3-10 cm. long, 7-10 mm. thick; glumes 5-6 mm. long, ciliate on the keel and pubescent on the side nerves; awn exerted, 2-5 mm.

Southeastern Alaska—Labr. and southward. Introduced from Eurasia. (Fig. 84.)

2. *A. glaucus* Less.

Glaucous Foxtail

A. occidentalis Scribn. & Tweedy.

Culms from long-creeping rhizomes, glaucous, up to 1 m. tall; panicle 15-40 mm. long, 8-12 mm. wide. This species has been confused with *A. alpinus* but has longer, more leafy, glaucous culms, longer and more cylindrical panicles, decidedly scabrous leaves.

Bering Sea—Yukon—Alta.—Mont.—Utah—Colo. Asia.

3. *A. alpinus* J. E. Sm.

Mountain Foxtail

Culms from creeping rhizomes, often decumbent at base, 1-6 dm. tall; sheaths glabrous, often inflated, longer than the internodes; blades 3-6 mm. wide; panicle 1-3 cm. long, 7-10 mm. wide; glumes 3-4 mm. long, very wooly; lemmas villous on upper portion, awn attached below the middle, usually exerted. Var. *stejnegeri* (Vasey) Hult. (*A. stejnegeri* Vasey) is characterized by narrow outwardly-curved and very acute glumes which are conspicuously longer than the lemmas. The awn is often longer and the panicle, 12-16 mm. thick. This is the form in the Aleutian Islands. Wet situations, Arctic coast—Central Alaska (Fig. 85.)

4. *A. aequalis* Sobol.

Short-awned Foxtail

Culms erect or spreading, 12–60 cm. long; leaves 1–4 mm. wide; panicle cylindric, 2–7 cm. long, 3–5 mm. wide; spikelets about 2 mm. long; awn scarcely exserted or exserted up to 1 mm.; anthers about 1 mm. long.

In water or wet soil, Aleutians, Bering Sea, and central Alaska east and south. Circumboreal. (Fig. 86.)

5. *A. geniculatus* L.

Marsh Foxtail

Similar to *A. aequalis*. Culms often rooting at the nodes; spikelets about 2.5 mm. long, the tip purple; anthers about 1.5 mm. long; awn of lemma about twice as long as the spikelet, exserted and giving the panicle a bristly appearance.

Introduced into southeastern Alaska. Native of Eurasia. (Fig. 87.)

6. POLYPOGON Desf.

Mostly decumbent annual grasses with spike-like panicles; spikelets 1-flowered, disarticulating below the glumes, leaving a short-pointed callus attached; glumes equal, awned from the tip; lemma shorter than the glumes, hyaline, usually with a short, straight awn. (Greek, many plus beard, referring to the bristly inflorescence.)

P. monspeliensis (L.) Desf.

Rabbit-foot Grass. Annual Beard-grass

Culms erect from a usually decumbent base, 15–50 cm. tall, sometimes depauperate or taller; leaves flat, 4–12 cm. long, 2–6 mm. wide; inflorescence dense, 2–15 cm. long, 1–2 cm. thick; glumes hispidulous, about 2 mm. long; awn 6–8 mm. long; lemma scarcely 1 mm long, its awn 0.5–1.5 mm. long.

Sparingly introduced as a weed. Native of Europe. (Fig. 88.)

7. PHLEUM L.

Perennial grasses with flat leaves; inflorescence a dense, cylindric, spike-like panicle; spikelets 1-flowered, compressed, disarticulating above the glumes; glumes equal, persistent, keeled, abruptly mucronate or awned; lemma shorter, hyaline, truncate, denticulate; palea narrow, nearly as long as the lemma. (Greek, a kind of reed.)

Panicle 1.5–3 times as long as broad1. *P. alpinum*
Panicle several times as long as broad.....2. *P. pratense*

1. *P. alpinum* L.

Mountain Timothy

Culms 15–50 cm. tall from a decumbent base; leaf-blades 2–10 cm. long, 3–8 mm. wide; inflorescence 1–5 cm. long, 7–12 mm. thick; glumes 3–4 mm. long exclusive of the awns which are about 2 mm. long.

Aleutian and Pribilof Islands and Pacific Coast regions of Alaska. Circumboreal and in South America. Our form averages taller and has shorter awns and more inflated upper sheaths than the European plant and has been described as var. *americanum* Fournier. (Fig. 89.)

2. *P. pratense* L.

Common Timothy

Culms erect, 5–10 dm. tall from a more or less swollen base; leaf-blades 5–25 cm. long, 4–8 mm. wide; inflorescence cylindric, 3–10 cm. long, 5–8 mm. thick; glumes 3–4 mm. long, scabrous, ciliate on the keel; awns about 1 mm. long.

A native of Eurasia and widely introduced in America, being cultivated for hay and pasture. Spontaneous in all Pacific Coast sections of Alaska and to some extent in the interior. (Fig. 90.)

8. PHIPPSIA R. Br.

Low annual tufted grass; leaves flat; panicle narrow; spikelets 1-flowered, disarticulating above the glumes; glumes unequal, minute, the first sometimes wanting; lemma thin, slightly keeled, 3-nerved, abruptly acute; palea somewhat shorter than the lemma. (John Constantine Phipps, 1744–1792, was an Arctic navigator.)

P. algida (Soland.) R. Br.

Phippsia

Glabrous; culms 3–15 cm. long; leaves soft, narrow, with boat-shaped tips; panicles 5–30 mm. long; spikelets 1–1.5 mm. long; grain oblong, enclosed in the lemma and palea.

Arctic Coast to St. Paul Island. Circumpolar. (Fig. 91.)

9. CINNA L.

Tall perennials with broad flat leaves and numerous spikelets in large, often nodding panicles; spikelets 1-flowered, the rachilla forming a stipe below the floret and produced beyond the palea as a minute bristle; glumes nearly equal, keeled, acute; lemma similar, nearly as long, 3-nerved, short-awned or awn-pointed; palea shorter, 1-nerved. (Greek name for some grass.)

C. latifolia (Trev.) Griseb.

Slender Reed-grass

Culms 5–15 dm. tall; leaf-blades 5–15 mm. wide; panicle 15–30 cm. long, the branches in verticils, capillary, flexuous, often drooping; spikelets about 4 mm. long; glumes hispidulous; lemma nearly as long as the glumes, hispidulous toward the apex; awn short.

Alaska Range and Pacific Coast districts of Alaska—Labr.—Newf.—N. Car.—Ill.—N. Mex.—Calif. (Fig. 92.)

10. CALAMAGROSTIS Adans.

Erect perennial grasses with paniced inflorescence; spikelets 1-flowered, the rachilla prolonged beyond the palea as a short, usually hairy bristle; glumes nearly equal, persistent, acute or acuminate; the lemma shorter with a basal ring of long hairs and a dorsal awn. Some of the species of this genus are very variable. This variability has resulted in much confusion regarding species and varieties. Some hybridization may have taken place. (Greek, signifying reed-grass.)

- 1A. Awn comparatively long, bent or geniculate, exserted.
 - 1B. Low-grown, less than 3 dm. tall.
 - 1C. Awn fixed at or below the middle of the lemma.....1. *C. deschampsoides*
 - 2C. Awn fixed near the apex of the lemma....2. *C. holmii*
 - 2B. Taller, stout grasses, 3–15 dm. tall.
 - 1C. 4–8 dm. tall, awn long-exserted3. *C. purpurescens*
 - 2C. 6–15 dm. tall, awn but slightly exserted...4. *C. nutkaensis*
- 2A. Awn not exserted, callus hairs as long or nearly as long as the lemma.
 - 1B. Panicle open, spreading.....5. *C. canadensis*
 - 2B. Panicle narrow or condensed.
 - 1C. Awn fixed at or above the middle of the lemma.....6. *C. neglecta*
 - 2C. Awn fixed below the middle of the lemma.
 - 1D. Leaves and panicle stiff7. *C. inexpansa*
 - 2D. Leaves and panicle soft8. *C. lapponica*

1. *C. deschampsoides* Trin.

Stems slender, erect, or decumbent at base, sheaths glabrous; leaves narrow, glabrous, mostly clustered at base, more or less involute, at least when dry; panicle erect, ovate, shining, 2–5 cm. long; spikelets 4–5 mm. long; glumes nearly equal, the lower 1-nerved, the upper 3-nerved, acute; lemma nearly equaling the glumes, bidentate, 5-nerved, awned at or below the middle; palea about equaling the lemma, bidentate; awn slightly longer than the lemma.

Western coast of Alaska, Eurasia, west coast of Hudson Bay. (Fig. 93.)

2. *C. holmii* Lange.

Holm Reed-grass

Relatively low-grown; upper ligules rudimentary; panicle open, small, rather dense; resembles *C. deschampsoides*, but the callus hairs are relatively shorter and the short, bent, or twisted awn is fixed near the apex of the lemma.

St. Paul Island, northeastern Asia, Nova Zembla, Arctic Siberia.

3. *C. purpurescens* R. Br.

Purple Reed-grass

C. yukonensis Nash.

Deyeuxia purpurescens (R. Br.) Schult.

Stems tufted, mostly 4–6 dm. tall; sheaths scabrous; leaves 2–4 mm. wide, flat or involute, scabrous; panicle dense, usually pinkish or purplish, somewhat spike-like, 2–12 cm. long; glumes 5–8 mm. long; lemma as long as the glumes, 4-toothed at apex, awned from near the base; hairs of the callus about one-third as long as the lemma.

Ssp. *arctica* (Vasey) Hult. [var. *arctica* (Vasey) Kearney] (*C. vaseyi* Beal) is a form growing up to 1 m. tall with hyaline abruptly pointed glumes. It occurs from the Alaska Peninsula to Japan.

Alaska—Victoria Land—Baffin Land—Greenl.—Que.—S. Dak.—Colo.—Calif.—N. Asia. (Fig. 94.)

4. *C. nutkaensis* (Presl) Steud.
C. aleutica Trin.

Pacific Reed-grass

Culms stout, 7–15 dm. tall; leaf-blades elongate, flat, becoming involute, gradually narrowed into a long point; panicle usually purplish, narrow, 12–30 cm. long, the branches stiffly ascending; glumes 5–7 mm. long, acuminate; lemma nearly as long as the glumes, indistinctly nerved; awn from below the middle, slightly geniculate, scarcely as long as the lemma; callus hairs and rachilla scarcely half as long as the lemma.

Along the coast, Aleutian Islands to Calif. (Fig. 95.)

5. *C. canadensis* (Michx.) Beauv.

Bluejoint

Culms tufted, 6–15 dm. tall, with numerous creeping rhizomes; leaf-blades elongate, flat, 2–8 mm. wide, scabrous; panicle 8–25 cm. long, open, usually purplish, the branches spreading; glumes 3–4 mm. long, acute, more or less scabrous; lemma nearly as long as the glumes, thin in texture, the awn delicate and extending to or slightly beyond its tip; callus hairs abundant, fully as long as the lemma.

The ssp. *langsdoerffii* (Link) Hult. [var. *scabra* (Presl) Hitchc.] [*C. langsdoerffii* (Link) Trin.] is the form most abundant in the coast districts. It has spikelets 4.5–6 mm. long, firm glumes which are hispid-ciliate on the keel, and the culms may attain a length of 23 dm.

Alaska—Greenl.—N. Car.—Kans.—Ariz.—Calif. (Fig. 96.)

6. *C. neglecta* (Ehrh.) Gaertn.

Narrow Reed-grass

Resembling *C. inexpansa* but averages smaller. Culms 3–8 dm. tall; leaves smooth or nearly so, narrow, often filiform; panicle 5–10 cm. long; spikelets 3–4 mm. long; glumes often nearly smooth except on the keel; callus hairs long but distinctly shorter than the lemma.

Var. *borealis* (Least.) Kearney differs from the type in its low growth, broad and short, flat leaves, and dark, purplish very acute glumes.

Bering Sea—Greenl.—Maine—Wis.—Utah—Ore. The variety is circumpolar.

7. *C. inexpansa* A. Gray.

Northern Reed-grass

C. hyperborea Lange.

Culms often scabrous below the panicle, 6–12 dm. tall, rhizomes present; leaf-blades loosely involute, scabrous, 2–4 mm. wide; panicle narrow, dense, 5–20 cm. long, the branches erect and spikelet-bearing from the base; glumes 3–4 mm. long, scabrous, abruptly acuminate; lemma about as long as the glumes, scabrous, green on the back with purplish tip; awn short, attached about the middle of the lemma or below; some of the rachilla hairs about reaching to the tip of the lemma.

Meadows and marshes, central Alaska—Greenl.—Maine—Mo.—N. Mex.—Calif. (Fig. 97.)

8. *C. lapponica* (Wahl.) Hartm.

Lapland Reed-grass

C. alaskana Kearney

Culms 4–9 dm. tall; leaves and panicle soft; panicle narrow, not very compact, often nodding; glumes purplish on back, brownish at the

tip, more or less evenly covered with scattered scabrous pubescence and a few comparatively large spinelike hairs on the middle of the keel; awn fixed near the base of the lemma, reaching about to the top of the glumes, longest callus-hairs as long as the lemma.

Bering Sea—Yukon—Mack.—Eurasia.

11. AGROSTIS L.

Delicate to moderately tall tufted grasses with paniculate inflorescence; spikelets small, numerous, 1-flowered; glumes obtuse, usually shorter and thinner than the glumes, awned or awnless; often hairy on the callus; palea small or obsolete. (Greek, referring to the field habitat of many of the species.)

- 1A. Palea evident, 2-nerved, at least half as long as the lemma.
 - 1B. Rachilla prolonged beyond the palea (*Podagrostis*).
 - 1C. Spikelets 3 mm. long 1. *A. aequivallis*
 - 2C. Spikelets 2 mm. long 2. *A. thurberiana*
 - 2B. Rachilla not prolonged.
 - 1C. Branches of panicle naked at the base 3. *A. tenuis*
 - 2C. Branches of panicle or some of them floriferous from base.
 - 1D. Panicles contracted, the branches appressed..... 4. *A. palustris*
 - 2D. Panicles open, the branches ascending.
 - 1E. Stems decumbent at base, rhizome wanting..... 5. *A. stolonifera*
 - 2E. Stems erect, rhizome present..... 6. *A. alba*
- 2A. Palea obsolete or minute.
 - 1B. Panicle narrow, contracted, branches spikelet-bearing from the base 7. *A. exarata*
 - 2B. Panicle open.
 - 1C. Panicle very diffuse 8. *A. scabra*
 - 2C. Panicle open but not diffuse.
 - 1D. Lemma awnless 9. *A. idahoensis*
 - 2D. Lemma awned.
 - 1E. Awn straight 10. *A. alaskana*
 - 2E. Awn geniculate 11. *A. borealis*

- 1. *A. aequivallis* Trin. Northern Bent-grass
Podagrostis aequivallis (Trin.) Scribn. & Merr.

Culms tufted, ascending from a spreading base, 2–6 dm. tall; leaves flat, 1–3 mm. wide; panicle usually purplish, 5–15 cm. long, the branches slender; spikelets 3 mm. or more long; glumes about equal, minutely scabrous beneath the tip of the keel; lemma about as long as the glumes, the palea nearly as long as the lemma; prolongation of the rachilla one-fifth to one-half as long as the lemma.

Western Aleutians south to Calif.

2. *A. thurberiana* Hitchc. Thurber redtop
Podagrostis thurberiana (Hitchc.) Hult.

Culms in small tufts, erect, 2-4 dm. tall; leaves about 2 mm. wide; panicles rather narrow, lax, 5-9 cm. long; spikelets about 2 mm. long, lemmas a little shorter than the glumes; palea about two-thirds as long as the lemma; prolongation of the rachilla short, hairy.

Aleutians—Central Alaska—Mont.—Colo.—Calif. (Fig. 98.)

3. *A. tenuis* Sibth. Colonial Bent. Rhode Island Bent

Culms slender, erect, 2-5 dm. tall; stolons short; leaves 1-3 mm. wide; panicle 3-10 cm. long, open, delicate; glumes 2 mm. long or less, sometimes slightly scabrous on the keel near the apex.

Introduced into southeastern Alaska. Native of Europe.

4. *A. palustris* Huds. Dense-flowered Bent
A. maritima Lam.

Culms tufted, erect, usually with a decumbent base, 2-4 dm. tall; leaves erect, rough on both surfaces, 4-8 cm. long, 1-3 mm. wide; panicle dense and compact, 3-10 cm. long; spikelets crowded, acute at both ends, lanceolate when closed, about 2 mm. long, on hispidulous pedicels thickened at apex; glumes acute, hispidulous on the upper part of the keel; lemma hyaline, palea one-half to two-thirds as long as the lemma.

Along the coast, Hyder—Calif. Also east coast and in Europe. (Fig. 99.)

5. *A. stolonifera* L.

Culms ascending, 2-7 dm. tall, with decumbent base and with stolons; leaves flat, 2-4 mm. wide, light or glaucous-green and scabrous; panicle 5-15 cm. long, pale or purple, somewhat open, some of the branches spikelet-bearing to the base; glumes acute, glabrous except the scabrous keel, 2-2.5 mm. long; lemma shorter than the glume, usually awnless, palea more than half as long as the lemma.

Central Alaska—N. Jer.—Ore. Also northern Europe. This form may be native. (Fig. 100.)

6. *A. alba* L. Redtop

Culms 3-15 dm. tall, erect or decumbent at base, with strong creeping rhizomes; leaves flat, 3-10 mm. wide, panicle reddish, 5-30 cm. long, lower branches verticillate, spreading; glumes acute, 2.5-3 mm. long; lemmas rarely awned.

Often used in lawn and pasture mixtures. Introduced. Native of Europe.

7. *A. exarata* Trin. Spike Redtop

Culms usually tufted, 2-12 dm. tall; ligule prominent, leaves flat, 1-8 mm. wide; panicle narrow, from somewhat open to spike-like, 10-25

cm. long; glumes acuminate or awn-pointed, 2.5–4 mm. long, scabrous, especially on the keel; lemma about 2 mm. long, often bearing an awn; palea about 0.5 mm. long.

Moist soil, common and variable, Aleutians—Man.—Nebr.—N. Mex.—Mex. and eastern Asia. (Fig. 101.)

Var. *purpurescens* Hult. 3–4 dm. tall, inflorescence dark purple. May be a hybrid with *A. alaskana*.

8. *A. scabra* Willd.

Ticklegrass

A. hiemalis Auct.

Culms tufted, 2–8 dm. long; leaves mostly basal, the blades usually narrow or even setaceous; panicle large and diffuse, 2–5 dm. long; spikelets crowded at the ends of the branchlets; glumes acute or acuminate, usually purplish; lemma shorter than the glumes, awnless or rarely awned; palea none. At maturity the panicle branches spread widely, and the whole panicle breaks away and rolls before the wind.

Var. *geminata* (Trin.) Hult. (*A. geminata* Trin.) Caespitose perennial; culms 1–3 dm. long; glumes about 3 mm. long; lemma usually awned. Alaska—Calif.

Var. *aristata* Hult. Spikelets about 2.4 mm. long; awns geniculate, exserted; panicle rather contracted. Central and southern Alaska.

Alaska—Newf.—Fla.—Mex.—Calif. (Fig. 102.)

9. *A. idahoensis* Nash.

Idaho Bent-grass

Culms slender, tufted, 1–3 dm. tall; leaves mostly basal, narrow; panicle loosely spreading, 5–10 cm. long, the flexuous branches capillary and minutely scabrous; spikelets 1.5–2 mm. long; lemma awnless; palea minute.

Fairbanks—Wash.—Mont.—N. Mex.—Calif.

10. *A. alaskana* Hult.

Alaska Redtop

A. melaleuca Am. auct.

Culms tufted, 1–5 dm. tall; panicles 5–20 cm. long, open, the branches in whorls; glumes dark purple, 2.5–3 mm. long, acute, smooth except the scabrous keel; lemmas white, about 2 mm. long, with a short, straight, variable awn; palea none. The var. *breviflora* Hult. has shorter and broader lemmas.

Coastal districts, Aleutians to southeastern Alaska. (Fig. 103.)

11. *A. borealis* Hartm.

Red Bent-grass

Culms tufted, 1–4 dm. tall; leaves mostly basal, 5–10 cm. long, 1–3 mm. wide; panicle 4–12 cm. long, the lower branches whorled and usually spreading; glumes 2.5–3 mm. long, acute, the lower usually slightly longer and more acute than the upper; lemma slightly shorter than the glumes, awned, the awn exserted; palea obsolete or nearly so.

Aleutians and Bering Sea Coast eastward. Circumboreal. (Fig. 104.)

12. ARCTAGROSTIS Griseb.

Perennial grasses; leaves flat; panicle contracted; spikelets 1-flowered; glumes unequal, membranous, acute; lemma longer than the glumes, obtuse; palea obtuse, 2-nerved; the lemma and palea strongly hispidulous. (Latin, Arctic and Agrostis.)

Spikelets 3–4.6 mm. long1. *A. latifolia*
Spikelets 2–3 mm. long2. *A. poaeoides*

1. *A. latifolia* (R.Br.) Griseb.

Arctagrostis

Culms 3–12 dm. tall, ligule prominent; blades 4–30 cm. long, 4–14 mm. wide; panicle rather narrow, somewhat open, 7–28 cm. long; glumes unequal, smooth, acute; lemma densely hispidulous, appearing acute in side view; palea similar to the lemma. A variable species, the typical form, seldom exceeding 5 dm. in height with purple spikelets 4 mm. or more long, is common in the Bering Sea and Arctic regions.

Var. *arundinacea* (Trin.) Griseb. [*A. arundinacea* (Trin.) Beal] is usually more than 5 dm. tall; spikelets usually less than 4 mm. long; usually purplish. This is the form common in central and southern Alaska. (Fig. 105.)

Var. *angustifolia* (Nash) Hult. (*A. angustifolia* Nash) has long, narrow, very flexible panicles with short branches bearing greenish spikelets and long, lax, bluish-green leaves. Dr. Hulten believes this form to be a hybrid between *A. latifolia* var. *arundinacea* and *A. poaeoides*.

This species is circumboreal.

2. *A. poaeoides* Nash.

Somewhat tufted and with running, branched rootstocks; culms 6–9 dm. tall, erect; sheaths striate, shorter than the internodes; ligule prominent; blades rough on both surfaces, 5–8 mm. wide; panicle about 15 cm. long, the main axis smooth, the pedicels rough; spikelets numerous, 2–3 mm. long; first glume 1-nerved, two-thirds as long as the 3-nerved second glume; lemma short, broad; the glumes, lemma, and palea all thin and translucent.

Central Alaska—Sask.—Man.

13. HOLCUS L.

Perennial grasses; leaf-blades flat; panicles contracted; spikelets 2-flowered, the pedicel disarticulating below the glumes, the rachilla curved and somewhat elongated below the first floret, not prolonged beyond the second floret; glumes about equal, longer than the lemmas; upper lemma bearing a short awn. (Old Latin name for a grass.)

H. lanatus L.

Velvet Grass

Plant grayish velvety-pubescent; culms erect, 3–7 dm. tall; leaves 4–8 mm. wide; panicles 6–15 cm. long, pale, purple-tinged; spikelets about 4 mm. long; glumes villous, hirsute on the nerves, the second broader than the first, 3-nerved; lemmas smooth and shining; awn hooklike.

A European grass sometimes cultivated and naturalized in southeastern Alaska. (Fig. 106.)

14. *ARRHENATHERUM* Beauv.

Tall perennial grasses; lower floret staminate, the upper perfect, the rachilla disarticulating above the glumes and produced beyond the upper floret; glumes rather broad and papery, the first 1-nerved, the second longer and 3-nerved, about the full length of the spikelet; lemmas 5-nerved, hairy on the callus, the lower bearing a short, minute, straight awn from near the tip; panicle rather dense. (Greek, for masculine plus awn, referring to the awned staminate floret.)

A. elatius (L.) Mert. & Koch.

Tall Oat-grass

Culms erect, smooth, 10–15 dm. tall; leaves flat, scabrous on both sides, 3–8 mm. wide; panicle shining, narrow, 1–3 dm. long, the short branches verticillate; spikelets 7–8 mm. long; lemmas scabrous.

Introduced in southeastern Alaska. Native of Europe. (Fig. 107.)

15. *SPHENOPHOLIS* Scribn.

Slender perennials; leaves flat; panicles narrow, shining; spikelets 2- or 3-flowered, the pedicel disarticulating below the glumes; the rachilla produced beyond the upper floret; glumes unlike, the first narrow, acute, 1-nerved, the second broadly ovate, 3–5-nerved, somewhat coriaceous, the margin scarious; lemmas scarcely nerved, awnless; palea hyaline, exposed. (Greek, wedge plus scale.)

S. intermedia (Rydb.) Rydb.

Slender Wedgegrass

Culms tufted, erect, 3–10 dm. tall; panicle erect and spike-like, somewhat interrupted and lobed; 5–20 cm. long; spikelets 2.5–3.5 mm. long.

Occurs at Manly Hot Springs. B. C.—Newf.—Fla.—Ariz. (Fig. 108.)

16. *KOELERIA* Pers.

Tufted grasses; leaf-blades narrow; panicle spike-like; spikelets 2–4-flowered, the rachilla disarticulating above the glumes and between the florets, prolonged beyond the perfect floret and often bearing a rudimentary floret at the tip; first glume narrow, 1-nerved, somewhat shorter than the second, which is wider and 3–5-nerved; lemmas somewhat scarious and shining, a little longer than the glumes, acute or short-awned. (George Ludwig Koeler was a German botanist.)

Culms and sheaths glabrous1. *K. yukonensis*
Culms and sheaths pubescent2. *K. cairnesiana*

1. *K. yukonensis* Hult.

Yukon Koeler-grass

Densely caespitose; culms 20–25 cm. tall, glabrous; basal leaves filiform, 0.2–0.3 mm. wide, glabrous, acute, ashy-green; cauline leaves wider, involute; spikelets about 7 mm. long, 3–5-flowered, long-pedicelled; glumes of about equal length; lemmas 5–7 mm. long, 5-nerved.

Known only from the upper Yukon district.

2. *K. cairnesiana* Hult.

Cairnes Koeler-grass

Culms 25–30 cm. tall, upper part pilose, slender; basal leaves filiform, about 0.5 mm. wide, glabrous; cauline leaves about 1 mm. wide, ciliate; panicles about 25 mm. long, dense, violet-tinged; spikelets 2-flowered, short-stipitate; glumes wide-lanceolate, scarious; lemmas about 3.5 mm. long, sparingly long-pilose, hyaline-margined.

Known only from the upper Yukon region.

17. *DANTHONIA* DC.

Tufted perennials; panicles spike-like; spikelets several-flowered; rachilla pubescent, extending beyond the florets; glumes about equal, broad, papery, acute, usually exceeding the florets; lemmas rounded on the back, the apex bifid, the lobes acute, often extending into slender awns, with a stout, flat, twisted, geniculate awn arising between them. (Etienne Danthione was a French botanist.)

Glumes pilose on the back1. *D. spicata*

Glumes glabrous on the back2. *D. intermedia*

1. *D. spicata* (L.) Beauv.

Poverty Oat-grass

Culms 2–5 dm. tall, slender; sheaths pubescent at the mouth; blades filiform to 2 mm. wide; panicle 2–5 cm. long, often 1-sided; spikelets 3–10; glumes 10–12 mm. long, acute; lemmas 4–5 mm. long, sparsely villous except the 2-toothed summit, the teeth acuminate or subsetaceous, 1.5–2.5 mm. long, flat.

Southeastern Alaska—Newf.—Fla.—N. Mexico—Ore. (Fig. 109.)

2. *D. intermedia* Vasey

Timber Oat-grass

Culms 1–5 dm. tall; blades subinvolute, or those of the stem flat; panicle 2–5 cm. long, few-flowered, purplish; branches appressed; glumes 12–16 mm. long, appressed-pilose along the margin below and on the callus, the teeth acuminate, aristate-tipped; awn 7–8 mm. long.

Extreme northwestern B. C.—Labr.—W. Newf.—Mich.—N. Mex.—Calif. Also Kamtchatka.

18. *AVENA* (Tourn.) L.

Annual or perennial grasses; spikelets usually large, in open or contracted panicles, 2–several-flowered, the rachilla bearded, disarticulating above the glumes and between the florets; glumes about equal, longer than the first floret and usually exceeding the upper one; lemmas indurate, except near the tip, 5–9-nerved, bidentate at tip, bearing a dorsal, bent, and twisted awn which is much reduced in the cultivated oat. (Old Latin name for the oat.)

A. fatua L.

Wild Oat

Annual; culm 3–12 dm. tall; leaves numerous, the blades flat, 1–3 dm. long, 5–15 mm. wide; panicle loose and open, 1–3 dm. long, the rachilla and

lower part of the lemmas with brownish hair; lemmas 12–20 mm. long; awn 15–40 mm. long.

Introduced in southeastern Alaska. (Fig. 110.)

A. sativa L., the cultivated oat, has usually smooth lemmas, 2-flowered spikelets, and the awn straight or wanting. It is occasionally found along roadsides.

19. *TRisetum* Pers.

Tufted perennial grasses; leaf-blades flat; inflorescence a spike-like, contracted, or open panicle; spikelets usually 2–3, rarely 4- or 5-flowered, the rachilla extending beyond the florets; glumes unequal, acute, entire at the apex, awnless, persistent; lemmas 2-toothed at the apex, the teeth acuminate and often bristle- or awn-pointed; awn often twisted and inserted below the apex on the back of the lemma. (Latin, referring to the awn and the two sharp teeth of the lemma.)

- | | |
|--|------------------------|
| 1A. Glumes nearly equal | 1. <i>T. spicatum</i> |
| 2A. Second glume much longer than the first. | |
| 1B. Panicle lax or drooping | 2. <i>T. cernuum</i> |
| 2B. Panicle dense | 3. <i>T. sibiricum</i> |
| 1. <i>T. spicatum</i> (L.) Richt. | Downy Oat-grass |
| <i>T. subspicatum</i> Beauv. | |
| <i>T. alaskanum</i> Nash. | |

Culms densely tufted, 15–50 cm. tall; sheaths and usually the blades puberulent; panicle dense and spike-like, often interrupted at the base, pale or more often purplish, 5–15 cm. long; spikelets 2–3-flowered, 5–6 mm. long; glumes somewhat unequal, acute or acuminate; lemmas 4–5 mm. long; the teeth setaceous; awn 5–6 mm. long, bent and twisted.

An arctic-alpine plant of cosmopolitan distribution and exceedingly variable. (Fig. 111.)

- | | |
|----------------------------|------------------|
| 2. <i>T. cernuum</i> Trin. | Nodding Trisetum |
|----------------------------|------------------|

Culms 6–12 dm. tall; leaves thin, flat, 1–2 dm. long, 4–8 mm. wide; panicle open, lax, drooping, 1–3 dm. long, the branches verticillate, flexuous, spikelet-bearing toward the ends; spikelets 6–12 mm. long, 2–3-flowered; lemmas 5–6 mm. long, the teeth setaceous, the awn twice as long as the lemmas.

Woods, southeastern Alaska—Ida.—Calif. (Fig. 112.)

- | | |
|------------------------------|-------------------|
| 3. <i>T. sibiricum</i> Rupr. | Siberian Trisetum |
|------------------------------|-------------------|

Culms erect, simple, smooth, 3–6 dm. tall; sheaths glabrous; leaves sometimes sparingly hairy; panicle contracted, 5–10 cm. long, brownish, shining; spikelets 5–10 mm. long, mostly 3-flowered; second glume much longer than the first; lemmas brown, about 5 mm. long; awn twisted and curved, 4–7 mm. long.

An Asiatic species. The plant here described and figured is the arctic

form which is found on the tundra in the northern part of the Bering Sea region. (Fig. 113.)

20. DESCHAMPSIA Beauv.

Mostly perennial grasses; panicles contracted or open; spikelets shining, pale or purplish, 2-flowered, the rachilla articulated above the glumes and prolonged beyond the florets; glumes nearly equal, persistent, keeled, acute; lemmas thin, almost hyaline, 2-4-toothed at the apex, bearded at the base, bearing a slender awn at or below the middle. (J. C. A. Loiseleur-Deslongchamps was a French physician and botanist.)

1A. Glumes extending beyond the lemmas.

1B. Annual1. *D. danthonioides*

2B. Perennial.

1C. Panicle very narrow2. *D. elongata*

2C. Panicle spreading3. *D. atropurpurea*

2A. Upper lemma extending to or beyond the glumes.

1B. Awn exserted, geniculate, twisted4. *D. flexuosa*

2B. Awn included or slightly exserted, nearly straight.

1C. Panicle open.

1D. Spikelets 6-7 mm. long5. *D. beringensis*

2D. Spikelets 3-5 mm. long6. *D. caespitosa*

2C. Panicle narrow7. *D. holciformis*

1. *D. danthonioides* (Trin.) Munro.

Annual Hair-grass

D. calycina Presl.

Stems slender, erect, 15-60 cm. tall; leaves few, short, narrow; panicle open, 7-20 cm. long, the capillary branches usually in twos, ascending, naked below; glumes 4-8 mm. long, 3-nerved, smooth except on the keel; lemmas smooth, shining, 2-3 mm. long, those of the base floret and the rachilla pilose; awns geniculate, 4-6 mm. long.

Probably introduced; Alaska—Mont.—Lower Calif. Also in Chile.

2. *D. elongata* (Hook.) Munro.

Slender Hair-grass

Culms densely tufted, erect, slender, 3-12 dm. tall; leaves 1-1.5 mm. wide, those of the basal tuft filiform; panicle very narrow, 1-3 dm. long, the capillary branches appressed; glumes 3-nerved, 4-6 mm. long, equaling or exceeding the florets; lemmas 2-3 mm. long, pilose at base, finely toothed at apex; rachilla pilose; awn inserted near the base of the lemma, 4-6 mm. long.

Alaska—Wyo.—Calif.—Mexico. Also in Chile. (Fig. 114.)

3. *D. atropurpurea* (Wahl.) Scheele.

Mountain Hair-grass

Vahlodea atropurpurea (Wahl.) Fr.

Culms loosely tufted, purplish at base, 3-8 dm. tall; leaves flat, 2-6 mm. wide; panicle loose, open, 5-10 cm. long; spikelets mostly purplish, broad; glumes about 5 mm. long, broad, exceeding the florets; lemmas

about 2.5 mm. long; awn attached at about the middle, bent. Our plant differs from the eastern American and European form in having wider leaves, upper part of inflorescence hairy, and shorter callus hairs which are one-third to one-half as long as the lemma. It was described as var. *paramushirensis* Kudo (ssp. *paramushirensis* Hult.) (var. *latifolia* Scribn.).

Var. *patentissima* has large panicle branches in verticels of 4-6, the lower up to 15 cm. long and bearing spikelets only on the distal fourth of their length.

The Pacific form occurs in the coastal districts of eastern Asia around the Bering Sea and south to Calif. (Fig. 115.)

4. *D. flexuosa* (L.) Trin.

Wavy Hair-grass

Stems densely tufted, erect, slender, 3-8 dm. tall; leaves numerous, mostly in a basal tuft, the sheaths scabrous, the blades involute, slender or setaceous, flexuous; panicle open, nodding, 5-12 cm. long, spikelets 4-5 mm. long, purplish or bronze; glumes 1-nerved, acute; lemmas scabrous, the callus hairs about 1 mm. long, awn attached near the base; awn geniculate, twisted, 5-7 mm. long.

Attu and Sitka—Greenl.—N. Car.—Okla. Also Eurasia, S. Am., E. Africa.

5. *D. beringensis* Hult.

Bering Hair-grass

D. bottnica Am. auct. not Trin.

Culms tufted, 3-12 dm. tall; leaves 1.5-4 mm. wide; panicle 7-20 cm. long, open, nodding, the branches scabrous; spikelets often 3-flowered, 6-7 mm. long; glumes about reaching to the top of the second floret, narrow, acute; lemmas 4-5 mm. long with long hairs at the base; palea scabrous. Hybridizes with *D. caespitosa* giving rise to intermediate forms.

Eastern Asia—Aleutian and Pribilof Islands—Ore. (Fig. 116.)

6. *D. caespitosa* (L.) Beauv.

Tufted Hair-grass

D. alaskana L. & M.

Culms in dense tufts, erect, 2-12 dm. tall; panicle 1-3 dm. long, loose, open, nodding; spikelets 3-5 mm. long, pale or purple-tinged, the rachilla joint half the length of the flower floret; glumes acute, 3-4.5 mm. long, lemmas 2.5-3.5 mm. long; awn about as long as, or slightly exceeding the lemma. A very variable species of circumboreal distribution. (Fig. 117.)

Hulten recognizes two forms as sufficiently distinct from the type to be entitled to recognition. Both are usually less than 3 dm. tall. Var. *glauca* (Hartm.) Sam. (*D. curtifolia* Scribn.) has filiform basal leaves which are bluish-green, the florets are very small, and the awn is fixed close to the base of the lemma.

Ssp. *orientalis* Hult. Basal leaves broad, flat, usually yellowish-green. This is the form found along the Arctic and Bering Sea Coasts.

7. *D. holciformis* Presl.

California Hair-grass

Culms caespitose, 5-12 dm. tall, relatively robust; leaves mostly basal, tightly folded or involute, firm, the lower ones long; panicle 10-25

cm. long, rather dense, purplish to brownish; spikelets 6–8 mm. long; glumes and lemmas scaberulous, the glumes about equaling or a little shorter than the spikelet; lemmas awned from below the middle; awns erect, exceeding the spikelet.

Along the coast, Ketchikan—Calif. (Fig. 118.)

21. BECKMANNIA Host.

Rather tall, more or less erect annuals; leaves flat; inflorescence consisting of numerous short, appressed or ascending spikes in a narrow somewhat interrupted panicle; spikelets 1–2-flowered, on one side of a slender rachis, falling off entire; glumes equal, saccate, 3-nerved; lemmas narrow, 5-nerved, about as long as the glumes. (Johann Beckmann, 1739–1811, taught natural history in St. Petersburg, now Leningrad.)

B. syzigachne (Steud.) Fern.

Slough-grass

B. erucaeformis Am. Auct.

Culms 3–10 dm. long; leaf-blades 4–8 mm. wide; panicle 10–25 cm. long; spikes crowded, 1–2 cm. long; spikelets 1-flowered, 3 mm. long; glumes transversely wrinkled, and with a deep keel; lemma with an acuminate apex protruding above the glumes.

Growing in mud or water, Alaska—Man.—Ill.—N. Mex.—Calif. Also in N. Y., Ohio, Asia. (Fig. 119.)

22. DACTYLIS L.

Tall perennial grass; panicle contracted, the spikelets crowded at the ends of the branches in unilateral clusters; spikelets 3–5 flowered; glumes unequal, acute, hispid-ciliate on the keel; lemmas compressed-keeled, mucronate, 5-nerved, ciliate on the keel. (Greek, finger, referring to the stiff branches of the panicle.)

D. glomerata L.

Orchard Grass

Culms 6–12 dm. tall, arising from large, dense tussocks; leaves flat, elongate, 2–8 mm. wide; panicles 5–20 cm. long, the branches spreading in anthesis, appressed at maturity, the rachis hispid; lemmas about 6 mm. long, mucronate or short-awned.

Introduced, native of Europe. (Fig. 120.)

23. DUPONTIA R. Br.

An arctic perennial grass; leaf-blades flat; panicle narrow; spikelets 2–4-flowered; glumes extending beyond the lemmas, membranous; lemmas membranous, entire, with a tuft of hair at the base. (J. D. Dupont was a French botanist.)

D. fischeri R. Br.

Dupontia

Culms smooth, erect, simple, 12–50 cm. tall; sheaths overlapping; blades 3–15 cm. long, 2–4 mm. wide; panicle usually contracted, 4–12 cm. long; spikelets mostly 2-flowered, 6–8 mm. long; glumes thin, generally acute, the first 1-nerved and usually shorter than the second which is

usually 3-nerved; lemmas 4–6 mm. long, 1-nerved or obscurely 3-nerved. The typical form with blunt-tipped glumes and subsericeous lemmas is a low-growing plant of the Arctic Coast. Ssp. *psilosantha* (Rupr.) Hult. (*D. psilosantha* Rupr.) is a taller growing form with acute glumes and glabrous lemmas. Intermediate forms occur where both types are found. Arctic and Bering Sea coasts. (Fig. 121.)

24. SCHIZACHNE Hack.

Rather tall perennials with simple culms and open, rather few-flowered panicles; spikelets several-flowered, disarticulating above the glumes and between the florets; glumes unequal, 3-nerved and 5-nerved; lemmas lanceolate, strongly 7-nerved, long-pilose on the callus, awned from just below the teeth of the strongly bifid apex; palea with softly pubescent submarginal keels, the hairs longer toward the summit. (Greek, *schizein*, to split, plus *achne*, chaff, referring to the teeth of the lemma.)

S. purpurescens (Torr.) Swallen.

False Melic

Avena striata Michx.

Culms erect from a loosely tufted, decumbent base, 5–10 cm. tall; blades flat, narrowed at the base, 1–5 mm. wide; panicles about 1 dm. long, the branches more or less drooping, bearing 1 or 2 spikelets; spikelets 20–25 mm. long; glumes purplish; lemmas about 1 cm. long, the awn as long or longer than the lemma.

Woods, southern Alaska—Newf.—Penn.—S. Dak.—Mont.—N. Mex.—B. C.—Siberia—Japan. (Fig. 122.)

25. COLPODIUM Trin.

Annual or perennial grasses; leaf-blades flat or almost setaceous; panicles diffuse, pyramidal, the branches capillary; spikelets 1–6 flowered, often colored, the rachilla disarticulating above the glumes and between the florets; glumes membranous or hyaline, 1–3-nerved or nerveless, obtuse or rather acute, unequal; lemmas with texture of the glumes, broad, obtuse, more or less 5-nerved, the lateral ones short or almost obsolete; palea almost as long as the lemma, hyaline.

Leaves 5–8 mm. broad1. *C. fulvum*
Leaves 1 mm. or less broad2. *C. wrightii*

1. *C. fulvum* (Trin.) Griseb.

Arctophila fulva (Trin.) Rupr.

A stout perennial, 2–9 dm. tall, rarely taller; culms and leaves smooth; blades flat, pungent-pointed or sometimes obtuse, 5–25 cm. long, 5–8 mm. wide; panicle open, ovoid, 8–15 cm. long, the branches drooping and bearing spikelets on the outer half; spikelets pedicellate, ovate or oblong, 5–6 mm. long, 4–6-flowered; first glume 1-nerved, second glume 3-nerved and about as long as the lemma; lemmas 3–5-nerved, 3–4 mm. long. The Arctic form of this species has the branches of the panicle ascending and

the spikelets are smaller, often only 1 or 2-flowered. This is the var. *effusum* (Lange) Polunin. There are intermediate forms.

Shallow water or mud, throughout most of our territory. Circumpolar. (Fig. 123.)

2. *C. wrightii* Scribn. & Merr.

Poa wrightii (Scribn. & Merr.) Hitchc.

Densely caespitose perennial, glabrous, 3–5 dm. tall; basal leaves rather short, linear, involute, about 5 cm. long, 1 mm. or less wide, those of the culm shorter; panicles open, purplish, 4–9 cm. long, the branches glabrous, the lower ones usually in pairs, spreading or ascending; spikelets 3- or 4-flowered, 6–8 mm. long; glumes unequal, the first 1.5–2.5 mm. long, the second 2.5–3.5 mm. long, obtuse, 3-nerved; lemmas lanceolate, rather obtuse, 4.5–5 mm. long, quite prominently 5-nerved, appressed silky-pubescent on the back toward the base, glabrous above.

Seward Peninsula and eastern Asia.

26. MELICA L.

Moderately tall perennial grasses with the base of the culm often swollen into a corm; spikelets 2–several-flowered, the rachilla disarticulating above the glumes and between the fertile florets, prolonged beyond the perfect florets and bearing at the apex 2 or 3 smaller empty lemmas each enclosing the one above; glumes somewhat unequal, thin, papery, scarious-margined, 3–5-nerved, sometimes nearly as long as the lower lemma; lemmas convex, membranous or rather firm, scarious-margined, usually awnless. (Italian for a sorghum, from the Greek, mel, honey.)

M. subulata (Griseb.) Scribn.

Alaska Onion-grass

Bromus subulatus Griseb.

Culms 6–12 dm. tall, mostly bulbous at the base; leaves thin, usually 2–5 mm. wide; panicle usually narrow, the branches appressed or sometimes spreading; spikelets narrow, 15–20 mm. long, loosely several-flowered; glumes narrow, obscurely nerved; lemmas prominently 7-nerved, narrowed to an acuminate point, awnless, the nerves more or less pilose-ciliate.

Apparently rare, Unalaska—Wash.—Mont.—Wyo.—Calif. [Fig. 124. (From a Wash. specimen).]

27. GLYCERIA R. Br.

Mostly perennial aquatic or marsh grasses with flat leaves and paniculate inflorescence; spikelets few–many-flowered, subterete or slightly compressed, the rachilla disarticulating above the glumes and between the florets; glumes unequal, short, usually scarious; lemmas broad, convex on the back, firm, scarious at the apex, 5–9-nerved; palea 2-keeled. (Greek, sweet, the seed of the type species being sweet.)

1A. Spikelets linear, more than 1 cm. long.

1B. Lemmas glabrous between the nerves.....1. *G. borealis*

2B. Lemmas scaberulous between the nerves2. *G. leptostachya*

2A. Spikelets less than 1 cm. long.

1B. Lemmas appearing to be 5-nerved3. *G. pauciflora*

2B. Lemmas plainly 7-nerved.

1C. Lemmas less than 2 mm. long4. *G. striata*

2C. Lemmas more than 2 mm. long.

1D. Culms usually more than 1 m. tall5. *G. grandis*

2D. Culms less than 1 m. tall6. *G. pulchella*

1. *G. borealis* (Nash) Batch.

Northern Manna-grass

Panicularia borealis Nash.

Culms glabrous, 6–15 dm. tall; sheaths smooth or slightly scabrous, keeled; blades flat, or usually folded, 1–2 dm. long, 2–4 mm. or more wide; panicle narrow, 2–4 dm. long, the branches and slender pedicels appressed; spikelets narrow, 10–15 mm. long, 6–12-flowered; glumes about 1.5 and 3 mm. long; lemmas 3–4 mm. long, 7-nerved, smooth except on the scabrous nerves.

In shallow water, central Alaska—Newf.—Conn.—Iowa—N. Mex.—Calif. (Fig. 125.)

2. *G. leptostachya* Buckl.

Davy Manna-grass

Culms 1–2 m. tall, rather stout or succulent; leaves flat, scaberulous on the upper surface, 4–10 mm. wide; panicle 2–6 dm. long, narrow with ascending branches; spikelets 1–2 cm. long, 8–14-flowered, often purplish; lemmas firm, broadly rounded toward the apex, about 3 mm. long, scaberulous both on the nerves and in between.

Wrangell—central Calif. (Fig. 126.)

3. *G. pauciflora* Presl.

Weak Manna-grass

Panicularia pauciflora (Presl.) Kuntze.

Culms 5–12 dm. long, from a decumbent, rooting base; leaves thin, flat, scabrous, mostly 10–15 cm. long, 5–15 mm. wide; panicle 10–25 cm. long, the branches usually more or less flexuous, the spikelets crowded on the upper half; spikelets 4–6 mm. long, 4–8-flowered; glumes short, broad; lemmas scabrous, about 2 mm. long, rounded and somewhat erose at the summit, prominently 5-nerved, the 2 marginal nerves short and inconspicuous.

Central Pacific Coast region of Alaska—S. Dak.—Colo.—N. Mex.—Calif. (Fig. 127.)

4. *G. striata* (Lam.) Hitchc. ssp. *stricta* (Scribn.) Hult.

Fowl Manna-grass

Panicularia nervata (Willd.) Kuntze var. *stricta* Scribn.

Culms 3–5 dm. tall, erect; blades 5–15 cm. long, 2–4 mm. wide; panicle about 1 dm. long; spikelets about 3 mm. long, 4–6-flowered; glumes about 0.5 and 1 mm. long; lemmas 1.5–2 mm. long, prominently 7-nerved, usually purplish, scarious tip inconspicuous; palea about as long as the lemma.

Central Alaska—Labr.—Newf.—N. Hamp.—Iowa—N. Mex.—Ariz.—

northern Calif. The type form is found in eastern America and extends to northern Florida.

5. *G. grandis* S. Wats.

American Manna-grass

G. maxima (Hartm.) Holmb. ssp. *grandis* (S. Wats) Hult.

Panicularia americana (Torr.) MacM.

Culm stout, glabrous, 1-2 m. tall; blades flat, 15-35 cm. long, 6-15 mm. wide; panicle 2-4 dm. long, very compound and spreading; spikelets 5-8 mm. long, 4-7-flowered; glumes scarious; lemmas purplish, about 2.5 mm. long.

Central Alaska—Pr. Edward Isl.—Tenn.—N. Mex.—Nev.—eastern Ore. (Fig. 128.)

6. *G. pulchella* (Nash) K. Schum.

Culms 4-6 dm. tall, stout, smooth; leaves crowded, blades 15-30 cm. long, 2.5-5 mm. wide, long-acuminate; panicle open, 15-30 cm. long, naked toward the base; spikelets 5-6 mm. long, 4-6-flowered; glumes brownish or purplish, scarious-margined, obtuse, much shorter than the lemmas; lemmas usually purplish, with broad hyaline margins above, strongly but minutely hispidulous, prominently 7-nerved, about 3 mm. long.

Central Alaska—Mack.—Alta.—B.C.

28. PUCCINELLIA Parl.

Spikelets several-flowered, usually terete or only slightly flattened, the rachilla disarticulating above the glumes and between the florets; glumes unequal; lemmas rounded on the back, usually 5-nerved, scarious and often erose at the tip; palea nearly equaling the lemma. Our species are tufted perennials with narrow or open panicles. Closely related to *Poa* and *Glyceria*, the species often being listed under one or the other of those genera. The treatment of the genus here followed is that of Mr. Jason R. Swallen in a paper recently published in the Journal of the Washington Academy of Sciences. (Puccinelli was an Italian botanist.)

1A. Anthers 1.8-2 mm. long 1. *P. phryganodes*

2A. Anthers less than 1.5 mm. long.

1B. Panicle branches distinctly scabrous.

1C. Anthers 0.3-0.5 mm. long 2. *P. hauptiana*

2C. Anthers 0.7-1.5 mm. long.

1D. Lemmas 3-4 mm. long, anthers 1.3-1.5 mm. long.....

..... 3. *P. grandis*

2D. Lemmas 2-3 mm. long, anthers less than 1 mm. long.....

..... 4. *P. borealis*

2B. Panicle branches glabrous or only very sparsely scabrous.

1C. Lemmas 3.5-4 mm. long, anthers 1.2-1.5 mm. long.

1D. Panicle branches ascending, elongate... 5. *P. glabra*

2D. Panicle branches stiffly spreading or reflexed.

1E. Spikelets 2- or 3-flowered 6. *P. triflora*

2E. Spikelets 4-8-flowered 7. *P. andersoni*

2C. Lemmas about 3 mm. long or less.

1D. Anthers 0.3–0.6 mm. long.

1E. Lemmas about 3 mm. long, culms up to 30 cm. tall..... 8. *P. alaskana* ..

2E. Lemmas 2–2.5 mm. long, culms less than 2 dm. tall..... 9. *P. paupercula*

2D. Anthers mostly 0.8–1 mm. long.

1E. Palea longer than the lemma13. *P. kamtschatica*

2E. Palea as long as or slightly shorter than the lemma.

1F. Panicle branches slender, usually closely appressed.... 12. *P. nutkaensis*

2F. Panicle branches stout, stiffly spreading or reflexed, naked in the lower half.

1G. Culms stout, erect from a decumbent base..... 10. *P. hulteni*

2G. Culms relatively slender, erect or ascending, densely tufted11. *P. pumila*

1. *P. phryganodes* (Trin.) Scribn. & Merr. Creeping Alkali-grass

Culms 5–15 cm. tall and in addition creeping decumbent culms resembling stolons, up to 4 dm. long; leaves short, rarely more than 4 cm. long and 1 mm. wide; panicle 1–4 cm. long, barely exerted, with comparatively few spikelets; spikelets 5–8 mm. long, 3–6-flowered; second glume nearly as long as the lemmas; lemmas 3–4 mm. long. Easily distinguished from the other species by the stolon-like culms and the long anthers. Fruits more freely in the northern parts of its range.

Saline or brackish flats along the coast, circumpolar. (Fig. 129.)

2. *P. hauptiana* Krecz. Haupt Alkali-grass

Densely tufted; culms 2–6 dm. tall; leaf-blades 1.5–2 mm. wide, loosely involute; panicle 4–16 cm. long, the branches ascending, spreading or reflexed; spikelets 3–5 mm. long, 3–6-flowered; first glume 1 mm. long; second glume 1.5 mm. long, mostly obtuse, laciniate; lemmas 2 mm. long or less, obtuse, laciniate.

Siberia—Alaska—Alta. (Fig. 130.)

3. *P. grandis* Swallen. Large Alkali-grass

Culms 4–9 dm. tall, erect or geniculate at the lower nodes; leaf-blades 0.5–3 mm. wide, flat or involute on drying; panicles 1–2 dm. long, the branches at first appressed but later spreading; spikelets appressed, tinged with purple, 8–15 mm. long, 5–12-flowered; first glume 2–3 mm. long, subacute; second glume more than 3 mm. long, obtuse; lemmas 3–4 mm. long, sparsely pilose at the base.

Salt marshes, southwestern Alaska—Calif. (Fig. 131.)

4. *P. borealis* Swallen. Northern Alkali-grass

Culms 3–7 dm. tall, erect from a decumbent base; leaf-blades 1–2 mm. wide, flat, glabrous below, scabrous above; panicles 1–2 dm. long, the

slender branches ascending to reflexed, in rather distant fascicles of 2-5; spikelets 4-6 mm. long, 4-6-flowered, usually purplish; first glume more than 1 mm. long; second glume less than 2 mm. long; lemmas 2-3 mm. long, minutely erose-ciliolate; anthers 0.6-0.7 mm. long.

Along coast and rivers, Alaska and Yukon. (Fig. 132.)

5. *P. glabra* Swallen.

Smooth Alkali-grass

Culms 25-40 cm. tall, erect or decumbent at the base; leaf-blades 1.5-3 mm. wide, flat or involute toward the tip, glabrous; panicles mostly 1-2 dm. long, the glabrous branches 4-10 cm. long, naked at the base; spikelets appressed, pale or purplish, 8-10 mm. long, 5-7-flowered; first glume 2-3 mm. long; second glume 3-4 mm. long, obtuse, minutely ciliolate; lemmas 3.5-4 mm. long, rather thin and shining, the nerves obscure.

Tidal flats, Alaska Peninsula—Kenai Peninsula—Kodiak Isl.

6. *P. triflora* Swallen.

Three-flowered Alkali-grass

Culms densely tufted, erect, 45-60 cm. tall; leaf-blades 1-1.5 mm. wide, soft, glabrous, flat or becoming involute; panicles 15-20 cm. long, the glabrous branches in rather distant fascicles of 2-5, naked at the base; spikelets appressed, deeply tinged with purple, 5-7 mm. long, 2 or 3-flowered; first glume 1.5-3 mm. long; second glume 2.5-4 mm. long; lemmas 3.5-4 mm. long, the nerves evident.

Shores of Cook Inlet.

7. *P. andersoni* Swallen.

Anderson Alkali-grass

Culms densely tufted, 15-40 cm. tall, erect from a decumbent base, shorter culms arising from the sides; leaf-blades 1-3 mm. wide, flat and soft; panicle 4-10 cm. long, the branches ascending or spreading, slightly scabrous, bearing 1-3 or up to 5 appressed spikelets, spikelets up to 1 cm. long, 4-8-flowered; first glume about 2 mm. long, acute; second glume 2.5-3 mm. long, acute; lemmas 3-3.5 mm. long, acute, erose, sparsely pilose at the base and lower part of the prominent nerves.

Known only from Point Lay on the Arctic coast. (Fig. 133.)

8. *P. alaskana* Scribn. & Merr.

Alaska Alkali-grass

Culms 6-30 cm. tall; leaf-blades 1-2 mm. wide, shorter than, to longer than the culms, usually soft and flat; panicle contracted, 2-9 cm. long; spikelets 4-6 mm. long, 3-5-flowered; first glume 1-1.5 mm. long, acute; second glume 2-2.5 mm. long, obtuse; lemmas 2.5-3 mm. long, rather prominently 5-nerved, appressed ciliolate on the nerves below.

Along the coast, Aleutian Islands and Bering Sea region. (Fig. 134.)

9. *P. paupercula* (Holm) Fern. & Weath.

Arctic Alkali-grass

Culms 5-20 cm. tall, longer than the leaves; leaf-blades 0.5-1 mm. wide, rather stiff and often curved; panicles 1-7 cm. long, few-flowered; spikelets 4-8 mm. long, 3-5-flowered; first glume 1-1.5 mm. long; second glume 1.5-2 mm. long; lemmas 2-2.5 mm. long, elliptic to ovate, erose.

Aleutians and eastern Asia through arctic America. (Fig. 135.)

10. *P. hulteni* Swallen.

Hulten Alkali-grass

Culms 3-4 dm. tall; leaf-blades 0.5-2.5 mm. wide, mostly involute; panicle 6-14 cm. long, the branches ascending, spreading, or reflexed; spikelets 5-6 mm. long, 3-5-flowered; first glume 1.5-2 mm. long, acute, slightly keeled; second glume 2-2.5 mm. long, slightly keeled and with strong lateral nerves; lemmas 2.5-2.8 mm. long, minutely toothed.

Beaches, Kodiak Island—southeastern Alaska. (Fig. 136.)

11. *P. pumila* (Vasey) Hitchc.

Small Alkali-grass

Culms erect or somewhat decumbent at the base, 1-3 dm. tall; leaf-blades flat, scaberulous, 1-2.5 mm. wide; panicles 2.5-15 cm. long, the short branches usually stiffly ascending to reflexed, bearing only one to a few spikelets; spikelets 5-7 mm. long, 3-6-flowered; first glume 1.5-2.5 mm. long; second glume 2.5-3 mm. long; lemmas about 3 mm. long, rather abruptly narrowed toward the apex, the nerves conspicuous, sparsely pubescent on the callus.

Along the coast, Kodiak Isl.—Vancouver Isl. (Fig. 137.)

12. *P. nutkaensis* (Presl) Fern. & Weath.

Pacific Alkali-grass

Culms 2-6 dm. tall, usually erect; leaf-blades 1-2 mm. wide, flat or loosely involute; panicle narrow, 5-20 cm. long, the few slender branches appressed; spikelets 6-9 mm. long, 4-9-flowered; glumes about 1.5 mm. and 2.5 mm. long; lemmas about 3 mm. long, narrowed to an obtuse apex which is erose or minutely fimbriate.

Along the coast, Aleutian Islands—Calif. (Fig. 138.)

13. *P. kamtschatica* Holmb. var. *sublaevis* Holmb.

Culms erect or somewhat decumbent at the base, 12-25 cm. tall; leaf-blades rather soft, smooth, flat or involute on drying, 2 mm. or less wide; panicle 4-10 cm. long, the branches narrowly ascending or later spreading, spikelet-bearing on the upper half; spikelets 3-4 mm. long, 3- or 4-flowered; first glume about half as long as the lemmas; second glume much broader and obtuse; lemmas about 2 mm. long, obtuse, glabrous.

Cold wet soil, Kamtchatka—southeastern Alaska.

29. POA L.

Grasses with contracted or open paniculate inflorescence and narrow, flat, folded, or involute leaves with a boat-shaped tip. Spikelets 2-6-flowered, flat, the rachilla disarticulating above the glumes and between the florets, the uppermost floret rudimentary; glumes acute, keeled, the first usually 1-nerved, the second 3-nerved; lemmas somewhat keeled, awnless, membranous, often scarious at the tip, 5-nerved, the nerves sometimes pubescent. (Greek for a grass.)

1A. Low grasses, usually 3 dm. tall or less.

1B. Plants annual 1. *P. annua*.

2B. Plants perennial.

1C. Spikelets 2-4-flowered.

- 1D. Stolons present.
 - 1E. Lower branches of panicle usually in twos.....12. *P. arctica*
 - 2E. Lower branches of panicle in twos-fours.....13. *P. irrigata*
- 2D. Plants tufted, not stoloniferous.
 - 1E. Coma at base of lemma copious.....15. *P. leptocomma*
 - 2E. Coma at base of lemma lacking.
 - 1F. Panicle branches slender, capillary19. *P. brachyanthera*
 - 2F. Panicle branches erect or appressed.
 - 1G. Spikelets 5-6 mm. long24. *P. glauca*
 - 2G. Spikelets 4-5 mm. long25. *P. rupicola*
- 2C. Spikelets 3-5-9-flowered.
 - 1D. Plants with stolons.
 - 1E. Plants dioecious 3. *P. confinis*
 - 2E. Plants with perfect flowers.
 - 1F. Culm flat with sharp edges 2. *P. compressa*
 - 2F. Culm terete or slightly flattened.
 - 1G. Panicle nodding, lemmas 6 mm. long..... 6. *P. turneri*
 - 2G. Panicle erect, lemmas shorter.....23. *P. komarovii*
 - 2D. Plants tufted, not stoloniferous.
 - 1E. Spikelets but little compressed, much longer than wide27. *P. sandbergii*
 - 2E. Spikelets decidedly compressed.
 - 1F. Leaves about 1 mm. wide.....20. *P. abbreviata*
 - 2F. Leaves 2-5 mm. wide.
 - 1G. Panicle compact21. *P. alpina*
 - 2G. Panicle open18. *P. merrilliana*
- 2A. Medium and tall perennial grasses more than 3 dm. high.
 - 1B. Plants with stolons.
 - 1C. Spikelets 2-4-flowered.
 - 1D. Glumes 6-8 mm. long, nearly as long as the spikelet..... 8. *P. macrocalyx*
 - 2D. Glumes decidedly shorter than the spikelet 4. *P. laxiflora*
 - 2C. Spikelets 3-5-9-flowered.
 - 1D. Culm strongly flattened, 2-edged 2. *P. compressa*
 - 2D. Culm terete or slightly flattened.
 - 1E. Lemmas 5-7 mm. long.
 - 1F. Leaves 4-8 mm. wide 7. *P. eminens*
 - 2F. Leaves narrower.
 - 1G. Lemmas lanate-pubescent on lower part.
 - 1H. Panicle nodding, spikelets green 6. *P. turneri*

- 2H. Panicle usually erect, spikelets violet or grayish 5. *P. lanata*
- 2E. Lemmas shorter.
- 1F. Lemmas lanate-pubescent on lower part 9. *P. norbergii*
- 2F. Lemmas smooth or scabrate, not lanate between the nerves.
- 1G. Panicle branches 2-4 (mostly 4) in lowest whorl 11. *P. eyerdamii*
- 2G. Panicle branches 3-5 (mostly 5) in lowest whorl 10. *P. pratensis*
- 2B. Plants tufted, without stolons.
- 1C. Spikelets 2-4-flowered.
- 1D. Lemmas about 4 mm. long, spikelets more than 5 mm. long.
- 1E. Spikelets much flattened 15. *P. leptocoma*
- 2E. Spikelets but little flattened 26. *P. hispidula*
- 2D. Lemmas about 3 mm. long or less, spikelets less than 5 mm. long.
- 1E. Panicle erect, narrow 24. *P. glauca*
- 2E. Panicle open.
- 1F. Lemmas glabrous or the keel slightly pubescent 14. *P. trivialis*
- 2F. Lemmas pubescent on the keel and marginal nerves.
- 1G. Glumes about as long as the first lemma 16. *P. nemoralis*
- 2G. Glumes shorter than the first lemma 17. *P. palustris*
- 2C. Spikelets 3-7-flowered.
- 1D. Spikelets 8-10 mm. long, 4-7-flowered 29. *P. ampla*
- 2D. Spikelets 6-8 mm. long, 3-5-flowered.
- 1E. Lemmas about 4 mm. long 28. *P. canbyi*
- 2E. Lemmas about 5 mm. long 22. *P. stenantha*

1. *P. annua* L.

Annual Bluegrass

Tufted, often decumbent, rooting at the nodes and forming mats; culms 5-25 cm. tall; leaf-blades soft, flat, lax, 1-3 mm. wide; panicle open, 3-7 cm. long; spikelets crowded, 3-6-flowered, about 4 mm. long; lemmas 2.5-3 mm. long, not webbed at base, 5-nerved, the nerves more or less pubescent on the lower half.

A weed, probably introduced from Europe but widespread and common in Alaska. (Fig. 139.)

2. *P. compressa* L.

Canada Bluegrass

Culms 15-60 cm. tall, pale bluish-green, solitary or a few together, decumbent at the base, strongly flattened, with long creeping rhizomes; leaves rather short, 1-4 mm. wide; panicle narrow, 3-7 cm. long, the short branches usually in pairs; spikelets 4-7 mm. long, 3-9-flowered;

glumes 2–3 mm. long; lemmas firm, 2–3 mm. long, the keel and marginal nerves sparingly pubescent; web at base scant or wanting.

Roadsides, central and southeastern Alaska. Introduced. Native of Eurasia. (Fig. 140.)

3. *P. confinis* Vasey

Dune Bluegrass

Plants dioecious, the two kinds similar; culms often geniculate at the base, usually less than 15 cm. tall, sometimes much taller; sheaths and involute blades smooth, firm, narrow; panicle narrow, contracted, 1–3 cm. long; spikelets 4–5 mm. long, 3–4-flowered; glumes unequal; lemmas 3 mm. long, scaberulous, sparsely webbed at base, the nerves faint.

Sand dunes and sandy meadows near the coast, B. C.—Calif. Reported as growing in southeastern Alaska.

4. *P. laxiflora* Buckl.

Loose-flowered Bluegrass

P. leptocoma elatior Scribn. & Merr.

Culms scabrous, 10–15 dm. tall; sheaths slightly scabrous, leaves lax, 2–4 mm. wide; panicle loose, open, 10–15 cm. long, nodding or drooping; lower branches in whorls of 3 or 4; spikelets 5–6 mm. long, 3–4-flowered; lemmas about 4 mm. long, webbed at base, sparsely pubescent on lower part of nerves.

Southeastern Alaska—western Ore.

5. *P. lanata* Scribn. & Merr.

Lanate Bluegrass

Culms erect, 25–40 cm. tall, from creeping rootstocks; leaves glaucous, scabrous, rather rigid, 2–4 mm. wide, acute and hooded at the apex; panicle open, 5–12 cm. long, the branches usually in twos; spikelets ovate, acute, purplish or brownish, 8–10 mm. long, 3–6-flowered; glumes acute, 3-nerved, scabrous on the keel; lemmas 6–7 mm. long, with broad hyaline margins, 5-nerved, obtuse, densely webby on the lower half, strigose above. Viviparous forms are frequent.

Aleutian Islands—Lake Athabasca—B. C. (Fig. 141.)

6. *P. turneri* Scribn.

Turner Bluegrass

Culms leafy, 2–4 dm. tall, from creeping rhizomes; leaves 3–5 mm. wide, 4–8 cm. long; panicles 6–9 cm. long, plumose and nodding; spikelets 7–10 mm. long, usually 3-flowered; glumes long and narrow; lemmas acute, about 6 mm. long, copiously pubescent on the keel and lower part of lateral nerves, less so between the nerves; coma at base copious. A beautiful grass.

Kenai Peninsula and Aleutian Islands. (Fig. 142.)

7. *P. eminens* Presl.

Large-flowered Spear-grass

P. glumaris Trin.

P. trinii Scribn. & Merr.

Culms glaucous, 4–10 dm. tall from creeping rootstocks; leaves thick, 4–8 mm. wide; panicle dense, 1–2 dm. long, contracted; spikelets 10–14 mm. long, 3–6-flowered; glumes up to 1 cm. long; lemmas 5–6 mm. long,

lacinate at the hyaline tip, pubescent at the base and lower part of the midrib and nerves.

All of our beaches except the high Arctic—Vancouver Island. Also Labr.—Que. (Fig. 143.)

8. *P. macrocalyx* Tr. & Mey.

Large-glumed Bluegrass

Culms smooth, up to 8 dm. tall; leaves flat, 2–5 mm. wide; panicle open, 1–2 dm. long, the lower branches in whorls of 3–5, spikelet-bearing near the ends; spikelets 6–9 mm. long, 2–4-flowered; glumes 3-nerved, narrow and long-acuminate, 6–8 mm. long, reaching to the apex of the second lemma; lemmas 5–6 mm. long, webbed at base, densely white-hairy on keel and marginal nerves.

Prince William Sound—Aleutian Islands—eastern Asia. (Fig. 144.)

9. *P. norbergii* Hult.

Norberg Bluegrass

Stoloniferous; culms 5–7 dm. tall, glabrous; culm leaves 3 or 4, 6–8 cm. long, 3.5–4 mm. wide, the upper surface minutely scaberulous, lower surface and margins scabrous; glumes 1-nerved, glabrous, glaucous, with wide hyaline margins, the apex tinged violet, the lower lanceolate, the upper ovate to acute lanceolate-ovate; lemmas minutely and densely scaberulous, sparsely long-pilose below, the keel and lateral nerves pilose for two-thirds their length.

Known only from Hoonah.

10. *P. pratensis* L.

Kentucky Bluegrass

Culms erect, 3–10 dm. tall, from creeping rhizomes; leaves flat or folded, 2–4 mm. wide, the basal often elongated; panicle open, the branches in fascicles of 3–5, ascending or spreading, naked below; spikelets 3–5-flowered, 3–6 mm. (mostly 4–5 mm.) long; lemmas about 3 mm. long, copiously webbed at base, silky pubescent on the keel and marginal nerves, the intermediate nerves prominent but glabrous. A very variable species and giving rise to many forms, some of which may be hybrids. The cultivated form has probably been introduced but the var. *alpigena* E. Fries [*P. alpigena* (E. Fr.) Hartm.] is native and in the far north has given rise to viviparous forms. On the average it is not so tall as the typical form, the culms arise singly and do not form mats, the leaves are narrower and the spikelets purplish. The var. *angustifolia* (L.) Kunth has been collected at Seward. It has basal shoots with long, narrow, involute leaves and was probably introduced.

The entire species is circumboreal. (Fig. 145.)

11. *P. eyerdamii* Hult.

Eyerdam Bluegrass

Rhizomes long-creeping; culms 5–7 dm. tall, slender, glabrous; leaves about 2 mm. wide, the margins and under surface smooth, the upper surface minutely scaberulous; panicles 10–15 cm. long; glumes 1–2-nerved, glabrous with hyaline margins and scaberulous keel; lemmas minutely scaberulous, webbed at base, the lower two-thirds of the keel and one-

third of the lateral nerves white-pilose, glabrous between; intermediate nerves indistinct, anthers 1.4–1.9 mm. long.

Kodiak Island and Prince William Sound region.

12. *P. arctica* R. Br.

Arctic Bluegrass

P. cenisia All.

Culms loosely tufted, erect from a decumbent base, 1–3 dm. tall; leaves mostly basal, flat or folded, 1–4 mm. wide, a single culm-leaf at about the middle of the culm; panicle open, 5–10 cm. long, the lower branches usually 2, spreading or even reflexed; spikelets 2–4-flowered, 5–8 mm. long; lemmas densely villous on keel and marginal nerves, pubescent on lower part between the nerves; webbing at base very variable. A form with short runners and with long cobweb-like coma at the base of the lemmas is the ssp. *williamsii* (Nash) Hult. (*P. williamsii* Nash). Another form with long slender culm and involute leaves has been described as ssp. *longiculmis* Hult. There are also viviparous forms.

Circumpolar. (Fig. 146.)

13. *P. irrigata* Lindm.

Stoloniferous; culms 12–40 cm. tall, solitary or a few at the ends of the stolons, glaucescent; leaves clustered on innovations at the base of the culm, less than 1 dm. long, culm-leaf short; panicle small, rather lax; spikelets 3.5–6 mm. long, 2–3-flowered, the short peduncles scabrous; glumes subequal, usually acuminate; lemmas with cobwebby base, the keel and lateral nerves pubescent; the keel of the glumes scaberulous and incurved toward the apex.

Central and southeastern coast of Alaska, probably introduced. Described from Sweden. Range not definitely known. (Fig. 147.)

14. *P. trivialis* L.

Rough Bluegrass

Culms erect from a decumbent base, 3–10 dm. tall, scabrous, at least toward the summit; leaves 2–4 mm. wide; panicle 6–15 cm. long, open, the branches spreading or ascending; spikelets 2 or sometimes 3-flowered, about 3 mm. long, lemmas about 2.5 mm. long, glabrous except the slightly pubescent keel and the prominent coma at the base, nerves prominent.

Aleutian Islands—southeastern Alaska—Newf.—Va.—S. Dak.—northern Calif. Introduced from Europe. (Fig. 148.)

15. *P. leptocoma* Trin.

Bog Bluegrass

P. paucispicula Scribn. & Merr.

Culms solitary or a few together, smooth, rather lax, 2–6 dm. tall; leaves flat, flaccid, 2–4 mm. wide; panicle lax, 5–10 cm. long; spikelets narrow, 5–6 mm. long, 2–4-flowered; glumes narrow, acuminate; lemmas 3.5–4.5 mm. long, narrowly lanceolate, acuminate. Var. *scabrinervis* Hult. has the keel and nerves of the lemmas scaberulous and the tuft at the base lacking.

Boggy places, southeastern Alaska—Utah—northern Mex.—Calif. (Fig. 149.)

16. *P. nemoralis* L.

Wood Bluegrass

Culms tufted, glabrous, 3-7 dm. tall; leaves rather lax, 1-2 mm. wide; panicle 4-12 cm. long, the branches spreading; spikelets 3-5 mm. long, 2-5-flowered; glumes narrow, sharply acuminate; lemmas 2-3 mm. long, faintly 5-nerved, sparsely webbed at base, silky-pubescent on keel and marginal nerves below.

Aleutian Islands—southeastern Alaska. Circumboreal. May have been introduced in our territory. (Fig. 150.)

17. *P. palustris* L.

P. triflora Gilib.

P. crocata Michx.

Culms loosely tufted, glabrous, with decumbent, flattened, purplish base, 3-15 dm. tall; leaves 1-4 mm. wide; panicle open, nodding, yellowish-green or purplish, 1-3 dm. long; spikelets 3-5 mm. long, 2-4-flowered; glumes acute; lemmas 2.5-3 mm. long, usually bronzed at the tip, webbed at base, villous on the keel and marginal nerves, intermediate nerves faint.

Wet or moist soil, Aleutian Islands and central Alaska south and east. Circumboreal. (Fig. 151.)

18. *P. merrilliana* Hitchc.

Merrill Bluegrass

P. glacialis Scribn. & Merr. not Stapf.

Densely caespitose, glabrous, 2-3 dm. tall; leaves rather broad, thin, flat, glabrous, ascending, pale green, 4-6 cm. long, 3-4 mm. wide; panicles 3-9 cm. long, the branches flexuous with 2 or 3 spikelets near the ends; spikelets about 7 mm. long, 5-flowered, broadly lanceolate; glumes unequal, acute, the first 3 mm. long, the second 1 mm. longer, 3-nerved; lemmas acute, 5-nerved, 4-5 mm. long, with very few hairs on keel and marginal nerves, not webbed at base or only slightly so.

Southeastern Alaska. (Fig. 152.)

19. *P. brachyanthera* Huft.

Caespitose, about 1 dm. tall; culm leaves 2 or 3, 1-3 cm. long, 1-1.5 mm. wide; panicle branches in twos, glabrous, bearing spikelets at the ends; spikelets 2-5-flowered; glumes about 2.5 mm. long, glabrous, wide-lanceolate, acute; lemmas with lateral nerves and keel short white-ciliate; palea as long as the lemma; anthers about 0.5 mm. long.

Aleutian Islands to Copper River.

20. *P. abbreviata* R. Br.

Low Spear-grass

Culms from close tufts, 15 cm. tall or less, erect, smooth; leaves crowded at the base, about 1 mm. wide; panicle contracted, 15-25 mm. long, branches short and erect; spikelets about 5 mm. long, 3-5-flowered; glumes acute, smooth; lemmas about 3 mm. long, obtuse, strongly pubescent all over.

Occurs on the Arctic Archipelago and has been reported from Alaska.

21. *P. alpina* L.

Alpine Bluegrass

Culms erect from a rather thick vertical crown, 1-3 dm. tall; leaves mostly basal, short, 2-5 mm. wide; panicle rather compact, 2-7 cm. long; spikelets broad, purplish, 5-6 mm. long, 3-5-flowered; glumes broad, acute, scabrous on the keel; lemmas 3-4 mm. long, villous on the keel and lateral nerves.

Alpine-arctic, Bering Strait east and south. Circumboreal. (Fig. 153.)

22. *P. stenantha* Trin.

Narrow-flowered Bluegrass

P. acutiglumis Scribn.

Culms tufted, 3-7 dm. tall; ligule prominent, as much as 5 mm. long; leaves flat or slightly involute, mostly basal; panicle lax, 5-13 cm. long, the branches in twos or threes; spikelets 3-5-flowered, 6-8 mm. long; glumes 3-nerved; lemmas about 5 mm. long, copiously pubescent on lower part of keel and marginal nerves, sparsely pubescent or glabrous between, intermediate nerves faint. Often the spikelets produce growing plants thus forming the var. *vivipara* Trin.

Aleutian Islands—Mont.—Colo.—Ore. (Fig. 154.)

23. *P. komarovii* Roshew.

Komarov Bluegrass

Subterranean stolons curved; culms not over 3 dm. tall, the base surrounded by a cylinder of hyaline sheaths; leaves relatively broad; panicle erect or nearly so, short-pyramidal, green or the scales brown-tipped; lemmas webbed at base and often with straight hairs between the veins below. Has the appearance of *P. alpina*. Produces viviparous forms.

Eastern Asia—Aleutian Islands—Arctic coast—southern Alaska.

24. *P. glauca* Vahl.

Glaucous Spear-grass

Culms tufted, erect, rigid, 15-50 cm. tall; uppermost leaf usually below the middle of the culm; leaves usually short, 1-2 mm. wide; panicle 3-8 cm. long, the branches erect or ascending; spikelets 2-4-flowered; 5-6 mm. long; glumes 3-nerved, glabrous, rough on the upper part of the keel; lemmas 3-4 mm. long, strongly pubescent on lower part of keel and marginal nerves, slightly pubescent on the base of the faint intermediate nerves, not webbed at base.

A common grass in most parts of our territory. Circumboreal. (Fig. 155.)

25. *P. rupicola* Nash.

Timberline Bluegrass

Culms tufted, erect, 10-25 cm. tall; leaf-blades erect or ascending, involute, 1-5 cm. long, 0.5-1.5 mm. wide; panicle 2-5 cm. long, purplish, the short branches ascending or appressed; spikelets 4-5 mm. long, 2-4-flowered; glumes 3-nerved, 2.5-3 mm. long; lemmas 3 mm. or more long, villous on the lower part of keel and marginal nerves, sometimes a few hairs on the internerves, no coma at the base.

Buffalo range of central Alaska, Mont.—N. Mex.—Calif. (Fig. 156.)

26. *P. hispidula* Vasey. Hispid Bluegrass

Tufted, culms 3-7 dm. tall; leaves 2-3 mm. wide; panicle somewhat contracted, 3-15 cm. long; spikelets about 3-flowered, 6 mm. long; glumes prominently nerved, lanceolate; lemmas narrow, acute, about 4 mm. long, the keel and marginal nerves with white lanate hairs, intermediate nerves sometimes slightly lanate, the intervening space scabrous with fine short hairs. The var. *aleutica* Hult. is a dwarf form with narrow leaves and small spikelets found in exposed spaces in the Aleutian Islands. This species also produces viviparous forms. It is probably responsible for the reports of *P. gracillima* Vasey from Alaska.

Bering Island and the Aleutians—southeastern Alaska. (Fig. 157.)

27. *P. sandbergii* Vasey. Sandberg Bluegrass

Culms up to 3 dm. or more tall form a dense tuft of short basal foliage; leaves soft, flat, folded or involute, about 2 mm. wide; panicle narrow, 2-10 cm. long, the branches short, erect or ascending; spikelets 5-7 mm. long; glumes about 4 mm. long; lemmas pubescent on the lower half, especially on the keel and near the margin.

As *P. secunda* this species has been reported as found in Yukon Territory—Nebr.—N. Mex.—Calif.

28. *P. canbyi* (Scribn.) Piper. Canby Bluegrass

Culms tufted, erect, smooth, 5-12 dm. tall; leaves scabrous above, leaves 1-2 mm. wide; panicle narrow, often compact, 10-15 cm. long; spikelets 6-8 mm. long, 3-5-flowered; glumes unequal, acute; lemmas about 4 mm. long, more or less crisp-pubescent on lower part of back.

Sandy or dry ground, Yukon—Que.—Isle Royal—Minn.—Colo.—Ariz.—eastern Ore.

29. *P. ampla* Merr. Big Bluegrass

Tufted; culms 8-12 dm. tall; leaves 1-3 mm. wide; panicle narrow, 10-15 cm. long, usually dense; spikelets 4-7-flowered, 8-10 mm. long; lemmas rounded on the back, smooth or minutely scaberulous, 4-5 mm. long.

Skagway and Yukon—Mont.—N. Mex.—Calif.

30. *FESTUCA* L.

Mostly tufted perennial grasses with paniculate inflorescence and 2-several-flowered spikelets with the rachilla disarticulating above the glumes and between the florets, the uppermost floret reduced; glumes narrow, acute, unequal; lemmas rounded on the back, more or less awned or sometimes awnless; palea scarcely shorter than the lemma. (An old Latin name for a weedy grass.)

1A. Basal leaves 3-10 mm. wide, flat, lax.

1B. Lemmas awnless or very short-awned1. *F. elatior*

2B. Lemmas with awns 5-20 mm. long2. *F. subulata*

2A. Basal leaves narrow, folded, or involute.

- | | |
|---|---------------------------|
| 1B. Low-growing, less than 3 dm. tall | 3. <i>F. brachyphylla</i> |
| 2B. Culms 3-5 dm. tall | 4. <i>F. rubra</i> |
| 3B. Culms 5-10 dm. tall | 5. <i>F. altaica</i> |

1. *F. elatior* L.

Meadow Fescue

Culms smooth, 5-12 dm. tall; leaves flat, 3-8 mm. wide, somewhat scabrous above; panicle mostly erect, 1-2 dm. long, contracted after flowering, branches spikelet-bearing nearly to the base; spikelets usually 6-8-flowered, 8-15 mm. long; glumes about 3 mm. and 4 mm. long; lemmas coriaceous, 5-7 mm. long, the apex hyaline, rarely short-awned. The var. *arundinacea* (Schreb.) Wimm. (*F. arundinacea* Schreb.) is a tall-growing form with usually 4-5-flowered spikelets.

Introduced at several places in Alaska. Native of Eurasia. (Fig. 158.)

2. *F. subulata* Trin.

Bearded Fescue

Culms 4-12 dm. tall; leaves flat, thin, green above, scabrous on both sides, 1-3 dm. long, 3-10 mm. wide; panicle loose, open, drooping, 15-40 cm. long, the branches in twos or threes, at length spreading or reflexed; spikelets loosely 3-5-flowered, 7-12 mm. long; lemmas somewhat keeled, scabrous toward the apex, 5-7 mm. long, attenuate into a scabrous awn 5-20 mm. long.

Woods, southeastern Alaska—Wyo.—Utah—northern Calif. (Fig. 159.)

3. *F. brachyphylla* Schult.

Alpine Fescue

Densely tufted; culms 10-25 cm. tall; leaves narrow, involute, 2-7 cm. long; panicle narrow and spike-like, 2-7 cm. long; spikelets 2-6-flowered, often purplish; lemmas 3-4 mm. long; awn scabrous, 2-3 mm. long. The type form has the leaves glabrous or nearly so. Ssp. *saximontana* (Rydb.) Hult. is a somewhat taller form with scabrous leaves, occurring in the interior.

Alpine-arctic situations throughout our territory. Circumboreal. (Fig. 160.)

4. *F. rubra* L.

Red Fescue

Culms more or less tufted, erect from a decumbent base, 3-10 dm. tall; leaves soft, smooth, usually involute, 7-15 cm. long; panicle 4-20 cm. long, usually contracted and narrow; spikelets 4-6-flowered, often purplish; lemmas 5-7 mm. long, smooth to scabrous or villous; awn 1-4 mm. long. A very variable group from which species, subspecies, and varieties have been described. Geographical races can be distinguished, but some of the characters such as the amount and nature of the pubescence of the lemma, do not follow the variation of other characters. The following names have been used for species, subspecies, and varieties, and the plants reported under these names in the genus *Festuca* refer to this species: *aucta*, *arenaria*, *barbata*, *glabrata*, *kitaiabeliana*, *lanuginosa*, *megastachya*, *mutica*, *richardsonii*, *subvillosa*.

A common and widely distributed species in our territory. Circumboreal. (Fig. 161.)

5. *F. altaica* Trin.

Rough Fescue

Plants forming dense tussocks; culms erect, smooth, 3–10 dm. tall; leaves narrow, involute, 15–30 cm. long; panicle loose and open, 1–2 dm. long; spikelets 10–15 mm. long, 3–5-flowered, usually suffused with purple; glumes unequal, nearly smooth; lemmas ovate, attenuate, finely and densely scaberulous, 7–11 mm. long, usually with a short awn.

Nearly throughout our territory and in eastern Asia. (Fig. 162.)

F. duriuscula L. [*F. ovina* L. var. *duriuscula* (L.) Koch], Hard Fescue, a native of Europe naturalized in America, has been found in Alaska. It resembles *F. brachyphylla* but is taller and has wider and firmer leaves.

F. megalura Nutt., the Western Six-weeks Fescue, a native of B. C.—Baja, Calif., has been found introduced in our area. It grows 2–6 dm. tall; has narrow panicles 7–20 cm. long, with appressed branches; spikelets 4 or 5-flowered; lemmas linear-lanceolate, scabrous on the back toward the apex, ciliate on the upper half and with awns 8–18 mm. long.

31. BROMUS L.

Spikelets several to many-flowered, the rachilla disarticulating above the glumes and between the florets; glumes unequal, acute, the first 1–3-nerved, the second 3–5-nerved; lemmas convex or keeled on the back, 5–9-nerved, 2-toothed at the apex, sometimes awnless but usually awned from between the teeth; palea shorter than the lemma; sheaths closed; leaf-blades flat; inflorescence a panicle of large spikelets. All species described here have been collected in Alaska, but it is not known if *B. brizaeformis*, *B. marginatus*, *B. racemosus*, and *B. secalinus* are able to maintain themselves. (Greek, an ancient name of the oat.)

1A. Lemmas compressed-keeled.

1B. Spikelets glabrous or slightly pilose.

1C. Panicle branches elongate, drooping.....1. *B. sitchensis*

2C. Panicle branches shorter, erect.....2. *B. aleutensis*

2B. Spikelets densely pilose3. *B. marginatus*

2A. Lemmas rounded on the back, not compressed-keeled.

1B. Perennials.

1C. Creeping rhizomes present.

1D. Lemmas glabrous4. *B. inermis*

2D. Lemmas pubescent, at least near the margin5. *B. pumpellianus*

2C. Creeping rhizomes wanting.

1D. Lemmas glabrous6. *B. ciliatus*

2D. Lemmas pubescent7. *B. pacificus*

2B. Annuals.

1C. Lemmas rounded above, teeth short.

1D. Panicle contracted, rather dense.

1E. Lemmas glabrous8. *B. racemosus*

2E. Lemmas pubescent9. *B. mollis*

2D. Panicle open, the branches spreading.

1E. Awn short or wanting10. *B. brizaeformis*

2E. Awn well developed.

1F. Sheaths glabrous11. *B. secalinus*

2F. Sheaths pubescent12. *B. commutatus*

2C. Lemmas narrow, the teeth long13. *B. tectorum*

1. *B. sitchensis* Trin.

Alaska Brome Grass

Perennial; culms smooth, 10–18 dm. tall, sheaths smooth; leaves smooth beneath, sparsely pilose above, 6–12 mm. wide; panicle large, lax, drooping, 25–35 cm. long; spikelets 2–3.5 cm. long, 4–12-flowered; lemmas scabrous, often hairy toward the base, about 12 mm. long; awn 5–10 mm. long.

Near the coast, southeastern Alaska—Wash. (Fig. 163.)

2. *B. aleutensis* Trin.

Aleutian Brome Grass

B. sitchensis Trin. var. *aleutensis* (Trin.) Hult.

Culms 5–10 dm. tall; leaves 5–10 mm. wide; panicle erect with stiffly ascending branches; spikelets 3–7-flowered; lemmas often 15 mm. long; and awn nearly 1 cm. long.

Near the coast, Aleutian Islands to Wash. (Fig. 164.)

3. *B. marginatus* Nees.

Large Mountain Brome Grass

Short-lived perennial; culms 6–12 dm. long, sheaths pilose; panicle erect, rather narrow, 1–2 dm. long; spikelets 25–35 mm. long, 7- or 8-flowered; glumes scabrous or scabrous-pubescent; lemmas coarsely pubescent, ovate-lanceolate, 11–14 mm. long; awn 4–7 mm. long.

Introduced, native of B. C.—S. Dak.—N. Mex.—Calif. (Fig. 165.)

4. *B. inermis* Leyss.

Smooth Brome

Culms erect, 6–12 dm. tall, from creeping rhizomes; leaves smooth, 5–10 mm. wide; panicle 1–2 dm. long, erect with whorled branches; spikelets 20–25 mm. long; first glume 4–5 mm. long, second glume 6–8 mm. long; lemmas 9–12 mm. long, obtuse, glabrous or scabrous, emarginate, usually awnless, occasionally with an awn 1 or 2 mm. long.

A cultivated grass from Europe that has become established in some places in our territory. (Fig. 166.)

5. *B. pumpellianus* Scribn.

Arctic Brome-grass

Culms 5–12 dm. tall, with creeping rhizomes; leaves 1–2 dm. long, 5–10 mm. wide, smooth beneath, scabrous or pubescent above; panicle 1–2 dm. long, rather narrow, with short, erect, or ascending branches; spikelets 2–3 cm. long, 7–11-flowered; lemmas 10–12 mm. long, 5–7-nerved, pubescent along the margin and across the back at the base; awn 2–3 mm. long. Var. *arcticus* (Shear) Porsild (*B. arcticus* Shear). Panicle purplish,

the branches spreading at flowering time, erect or ascending at maturity; spikelets 2–4.5 cm. long, 6–14-flowered; glumes and lemmas coarsely pubescent, the lemmas 5-nerved, 12–14 mm. long. Var. *villosissimus* Hult. Glumes and lemmas densely villous-gray; leaves and sheaths often also villous.

Northern Bering Sea region—Ida.—Black Hills—Colo. (Fig. 167.)

6. *B. ciliatus* L.

Fringed Brome

B. richardsonii Link

Culms moderately robust, 7–12 dm. tall; sheaths often more or less pubescent; leaves up to 1 cm. wide; panicle 15–25 cm. long, open, the branches drooping; spikelets 2–3 cm. long, 5–10-flowered; lemmas nearly glabrous on the back, pubescent along the lower half to three-quarters of the margins, about 12 or 13 mm. long; awn 3–5 mm. long.

Central Alaska—Newf.—N. Jer.—Tenn.—Texas—Calif.—northern Asia. (Fig. 168.)

7. *B. pacificus* Shear.

Pacific Brome-grass

Culms stout, erect, 10–15 dm. tall, pubescent at the nodes; sheaths more or less retrosely pilose; leaves sparsely pillose above, 8–14 mm. wide; panicle very open, 10–25 cm. long, the branches slender, drooping; spikelets 20–25 mm. long, pubescent; lemmas 11–12 mm. long; awn 4–6 mm. long.

Along the coast, southeastern Alaska—Ore. (Fig. 169.)

8. *B. racemosus* L.

Smooth-flowered Soft Cheat

Resembling *B. mollis* but the panicle usually more open and lemmas glabrous or scabrous.

An European annual species adventitive in central Alaska and Yukon.

9. *B. mollis* L.

Soft Chess

Softly pubescent throughout; culms erect, 2–8 dm. tall; panicle contracted, the branches erect or ascending, 5–10 cm. long; glumes broad; lemmas broad with hyaline margins and tip, obtuse, 7-nerved, bidentate, 7–9 mm. long; awn 4–8 mm. long. This species has been reported as *B. hordaceus* L.

An introduced annual weed, native of Europe. (Fig. 170.)

10. *B. brizaeformis* Fisch. & Mey.

Rattlesnake Grass

Culms 3–6 dm. tall; sheaths and blades pilose-pubescent; panicle 5–15 cm. long, lax, secund, nodding; spikelets 15–25 mm. long, about 1 cm. wide, flat; lemmas about 1 cm. long, very broad, inflated, smooth, with broad scarious margins, awnless or nearly so.

An European species that has been collected at Seward and Nome.

11. *B. secalinus* L.

Chess or Cheat

Culms erect, 3–6 dm. tall; sheaths smooth, panicle nodding, 7–12 cm. long, the lower branches 3–5, unequal, drooping; spikelets 1–2 cm. long,

6-8 mm. wide; lemmas 6-8 mm. long, the margin strongly involute at maturity; awns usually 3-5 mm. long.

Often a weed in fields of grain. Native of Europe.

12. *B. commutatus* Schrad.

Hairy Chess

Resembles *B. secalinus*, but the sheaths are pilose with short retrose hairs; lemmas at maturity are less plump and the awn straight and usually longer.

An introduced weedy grass that is native of Europe.

13. *B. tectorum* L.

Downy Chess

Culms 3-6 dm. tall, glabrous; sheaths and blades more or less pubescent; panicle 6-15 cm. long, open, the branches slender and drooping, somewhat one-sided; spikelets nodding, 12-20 mm. long exclusive of awns; glumes villous; lemmas villous or pilose, 10-12 mm. long, the teeth 2-3 mm. long; awn straight, 12-18 mm. long.

An introduced weed that is becoming a pest in some localities. Native of Europe. (Fig. 171.)

Secale cereale L., the cultivated rye, has infrequently been found along roadsides and in old fields where it sometimes persists for several years. It is doubtful if it can maintain itself indefinitely.

Triticum aestivum L., the common wheat, like rye, is sometimes found along roadsides. It cannot be considered as really established and therefore a part of our flora.

32. *LOLIUM* L.

Spikelets several-flowered, solitary, sessile, placed edgewise to the rachis, one edge fitting into the alternate concavities; first glume wanting (except in terminal spikelet), the second outward, 3-5-nerved, equaling or exceeding the second floret; lemmas rounded on back, 5-7-nerved. (*Lolium*, an old Italian name for darnel.)

Glume shorter than the spikelet.

Lemmas awned1. *L. multiflorum*

Lemmas awnless or nearly so2. *L. perenne*

Glume as long as or longer than the spikelet3. *L. tremulatum*

1. *L. multiflorum* Lam.

Italian Ryegrass

A short-lived perennial with culms 4-10 dm. tall; spikes 8-30 cm. long, flat; spikelets 10-20-flowered, up to 25 mm. long; lemmas 7-8 mm. long, at least part of them awned.

Introduced. Native of Europe. (Fig. 172.)

2. *L. perenne* L.

English Ryegrass

Culms 3-6 dm. tall; spikelets 6-10-flowered; lemmas 5-7 mm. long, awnless. Often used in lawn grass mixtures.

Introduced. Native of Europe.

3. *L. temulatum* L.

Darnel

Annual, culms 6–9 dm. tall; leaves 3–6 mm. wide; spike 15–20 cm. long; glume about 25 mm. long, as long as or longer than the 5–7-flowered spikelet.

Has been collected near Dawson and at St. Michael, but it is doubtful if it has become established. Native of Europe.

33. AGROPYRON Gaertn.

Perennial grasses; leaves flat or involute; inflorescence a terminal spike; spikelets several-flowered, usually solitary, compressed, placed flat-wise at each joint of the rachis, the rachilla disarticulating above the glumes and between the florets; glumes firm; lemmas convex on the back, rigid, 5–7-nerved, acute or awned at the apex; palea nearly as long as the lemma.

A difficult genus with many variable forms probably due to hybridization. (Greek, wild and wheat, referring to their growth in wheat fields.)

1A. Plants with creeping rhizomes

1B. Glumes rigid, tapering to a short awn.....1. *A. smithii*

2B. Glumes not rigid, acute or abruptly short-awned.

1C. Lemmas glabrous2. *A. repens*

2C. Lemmas pubescent3. *A. yukonense*

2A. Plants tufted, without creeping rhizomes.

1B. Lemmas awnless or awn-tipped only.

1C. Nodes of culm finely appressed-pilose4. *A. alaskanum*

2C. Nodes of culm glabrous.

1D. Lemmas pubescent.

1E. Spikelets very narrow5. *A. sericeum*

2E. Spikelets comparatively broad6. *A. latiglume*

2D. Lemmas glabrous7. *A. trachycaulum*

2B. Lemmas awned.

1C. Awns straight8. *A. subsecundum*

2C. Awns divergent9. *A. spicatum*

1. *A. smithii* Rydb.

Western Wheat-grass

Culms usually glaucous, 3–6 dm. or more tall; leaves firm, stiff, scabrous; striate, 2–4 mm. wide, sharp-pointed, becoming involute on drying; spikes 6–15 cm. long, the rachis scabrous on the angles; spikelets rarely 2 at a node, 5–10-flowered, 1–2 cm. long; glumes rigid, tapering into a short awn, faintly nerved, 7–12 mm. long; lemmas about 1 cm. long, acuminate, mucronate or short-awned.

Central Alaska, probably introduced from the western states. (Fig. 173.)

2. *A. repens* (L.) Beauv.

Quackgrass

Culms 5–15 dm. tall, from long-jointed running rootstocks; leaves flat, smooth beneath, rough above, mostly 5–10 mm. wide; spike 5–15 cm. long,

the rachis scabrous on the margins; spikelets 3-7-flowered, 10-15 mm. long; glumes strongly 3-7-nerved, acute or awn-pointed; lemmas about 8 mm. long, acute or awned, the awn when present may approach that of the lemma in length.

Central Alaska south and east. A native of Eurasia and extensively introduced in North America. (Fig. 174.)

3. *A. yukonense* Scribn. & Merr.

Yukon Wheat-grass

Culms glabrous, 4-8 dm. tall, from creeping rhizomes; leaves 2-6 mm. wide, flat or involute; spikes 5-12 cm. long; spikelets closely imbricate, 4-8-flowered, 10-15 mm. long; glumes acute, 3-nerved, pilose; lemmas villous, about 8 mm. long, acute or short-awned.

Upper and central Yukon River valley. (Fig. 175.)

4. *A. alaskanum* Scribn. & Merr.

Alaska Wheat-grass

Culms glabrous, erect, 4-9 dm. tall, the nodes pubescent; leaves flat or involute, 4-7 mm. wide, scabrous on both surfaces; spike 6-10 cm. long, rather slender; spikelets sometimes 2 at a node, 15-20 mm. long, 4-6-flowered, exceeding the scabrous internodes of the rachis; glumes 6-8 mm. long, oblanceolate; lemmas 8-10 mm. long exclusive of the short awn, lanceolate, hispid with short stiff hairs along the sides but the pubescence variable. The arctic variety *arcticum* Hult. has the glumes and lemmas hispid to pilose.

Matanuska—Arctic coast—upper Yukon district. (Fig. 176.)

5. *A. sericeum* Hitchc.

Culms tufted, 4-12 dm. tall; leaves flat, rather long, mostly 4-9 mm. wide; spikes 6-25 cm. long; spikelets remote to loosely imbricated, usually narrow, 3-7-flowered, 15-22 mm. long; glumes mostly 3-nerved, acute or short-awned, glabrous or slightly pubescent, 6-15 mm. long; lemmas short-villous, acuminate or short-awned, 8-12 mm. long; rachilla pubescent.

Nome—Matanuska—upper Yukon valley. (Fig. 177.)

6. *A. latiglume* (Scribn. & Sm.) Rydb.

A. violaceum (Hornem.) Lange var. *latiglume* Scribn. & Sm.

Culms loosely tufted, curved or geniculate below, 2-7 dm. tall; leaves flat, rather short, 3-5 mm. wide; spike 3-7 cm. long; spikelets imbricate, 10-18 mm. long, 3-5-flowered; glumes rather broad, flat or rounded, 9-12 mm. long; lemmas pubescent, acute or short-awned, 7-11 mm. long.

Most of our territory; circumpolar. (Fig. 178.)

7. *A. trachycaulum* (Link) Hitchc.

Slender Wheat-grass

A. angustiglume Nevski.

A. pauciflorum (Schwein.) Hitchc.

A. tenerum Vasey

Culms tufted, 6-12 dm. tall; leaves mostly 2-4 mm. wide; spikes slender, 5-20 cm. long; spikelets from rather remote to closely imbricate,

12–15 mm. long, 2–5-flowered; glumes firm, acute to awn-pointed, 10–12 mm. long; lemmas 8–10 mm. long, acute or short-awned. Very variable and our most common species.

Above the Arctic Circle in Alaska—Labr.—W. Va.—Mo.—N. Mex.—Calif. (Fig. 179.)

8. *A. subsecundum* (Link) Hitchc. Bearded Wheat-grass

A. caninum Am. auct. not (L.) Beauv.

A. richardsonii Schrad.

Culms tufted, erect, 3–10 dm. tall; leaves scabrous, flat, 3–8 mm. wide; spike erect or slightly nodding, 6–15 cm. long, rather dense; spikelets 12–15 mm. long, 3–5-flowered; glumes unequal, acuminate or awn-pointed, 4–7-nerved; lemmas scabrous, 8–12 mm. long, with a nearly straight awn 1–2 cm. long.

Central Alaska—Newf.—Md.—Nebr.—N. Mex.—Calif. (Fig. 180.)

9. *A. spicatum* (Pursh.) Scribn. & Sm. Bluebunch Wheat-grass

Culms tufted, rigid, 6–10 dm. tall; leaves 1–4 mm. wide; spike slender, 8–15 cm. long; spikelets distant, often shorter than the internodes of the rachis, 3–8-flowered; glumes acute but not awned; lemmas 8–10 mm. long, terminating in a bent awn 10–25 mm. long, rachilla scabrous.

Central Alaska—Mich.—N. Mex.—Calif. (Fig. 181.)

34. HORDEUM L.

Leaf-blades flat; inflorescence a terminal spike; spikelets 1-flowered, usually in threes at each joint of the rachis, the middle spikelet sessile and perfect, the lateral usually pedicelled and imperfect; glumes narrow, often subulate and awned, rigid, standing in front of the spikelet; lemmas lanceolate, rounded on the back, tapering to a usually long awn. (Latin name for barley.)

Awn less than 12 mm. long1. *H. brachyantherum*

Awn 15–35 mm. long2. *H. caespitosum*

Awn 4–7 cm. long3. *H. jubatum*

1. *H. brachyantherum* Nevski. Meadow barley

H. boreale Scribn. & Sm. not Gavdoger.

H. nodosum Auct. in part.

Tufted perennial; culms 5–10 dm. tall; leaves 4–8 mm. wide, scabrous; spikes slender, 2–8 cm. long; glumes all setaceous, 8–15 mm. long; lemma of central spikelets 7–8 mm. long; awn exceeding the glumes; lemmas of lateral spikelets considerably reduced.

Coastal regions of Alaska and northeastern Asia. Also Labr. and Newf. (Fig. 182.)

2. *H. caespitosum* Scribn. Bobtail barley

Culms 3–10 dm. tall; glumes and awns 15–35 mm. long. In nearly all

characters this form is intermediate between *H. brachyantherum* and *H. jubatum* and since it is found only in the coastal sections where both these species occur, it is probably a hybrid between them. (Fig. 183.)

3. *H. jubatum* L.

Squirrel-tail barley

Tufted perennial; leaves 2–4 mm. wide, scabrous; spikes nodding, 5–10 cm. long; glumes awnlike, 4–7 cm. long; lemma of central spikelet 6–8 mm. long with awn as long as the glumes; lemmas of lateral spikelets reduced almost to a short awn.

Open ground, all of Alaska except the Arctic—Labr.—Newf.—Md.—Mo.—Mex. Also Asia. (Fig. 184.)

Hordeum vulgare L., the annual, cultivated barley, like other grains, is sometimes found adventitive along roadsides.

35. ELYMUS L.

Perennials with spicate inflorescence; spikelets 2–6-flowered, usually 2 but sometimes 1 or 3 at each node of the rachis, the rachilla disarticulating above the glumes and between the florets; glumes equal, forming an apparent involucre to the cluster, rigid, narrow to subulate; lemmas oblong to lanceolate, rounded on the back, 5-nerved, usually awned; palea a little shorter than the lemma, 2-keeled. (Greek, an ancient name for a kind of barley.)

1A. Culms from creeping rhizomes.

1B. Spikelets 10–15 mm. long1. *E. innovatus*

2B. Spikelets 12–25 mm. long.

1C. Glumes nearly as long as the spikelet2. *E. mollis*

2C. Glumes much shorter than the spikelet3. *E. aleuticus*

2A. Culms tufted, no creeping rhizomes.

1B. Lemmas awnless or nearly so4. *E. virescens*

2B. Lemmas awned.

1C. Awns curved, divergent5. *E. canadensis*

2C. Awns straight.

1D. Rachis tardily disjointing6. *E. macounii*

2D. Rachis continuous.

1E. Lemmas glabrous or scabrous7. *E. glaucus*

2E. Lemmas sparsely long-hirsute on the edge

.....8. *E. hirsutus*

1. *E. innovatus* Beal.

Downy Rye-grass

Culms erect from horizontal rhizomes, 4–9 dm. tall; leaves rather rigid, flat or involute, 2–8 mm. wide; spike rather dense, 4–10 cm. long; spikelets 10–15 mm. long, 3–6-flowered; the narrow glumes and the lemmas densely purplish or grayish villous, the lemmas 8–10 mm. long with awns 1–4 mm. long.

Grassy flats, central Alaska—mouth of the Mackenzie River—S. Dak.—Wyo.—B. C. (Fig. 185.)

2. *E. mollis* Trin. Beach Ryegrass
E. arenarius L. ssp. *mollis* (Trin.) Hult.
E. villosissimus Scribn.

Culms stout, glaucous, erect, from a widely creeping rhizome, 6-20 dm. tall; sheaths smooth, leaves smooth or scabrous above, 7-12 mm. wide; often involute on drying; spikes erect, dense, thick, soft, 7-25 cm. long; glumes scabrous or pubescent, 12-24 mm. long, acuminate, nearly as long as the spikelet; lemmas scabrous to villous-pubescent, acuminate or mucronate. Attu baskets are made of the fibers from the leaf of this species. In the far north it becomes much dwarfed.

Sandy beaches, Alaska—Calif.; with closely related species, circum-boreal. (Fig. 186.)

3. *E. aleuticus* Hult. Aleutian Rye-grass

Culms 6-7 dm. tall, arising from elongated creeping rhizomes; ligule 0.7 mm. long, ciliate, spikes 10-15 cm. long; spikelets 3-5-flowered, about 25 mm. long; glumes lanceolate, 3-5-nerved, about 1 cm. long, scarious wing-margined, acute, sparsely pilose; lemmas 15-20 mm. long, acute, 5-nerved, short-pilose; awn 2-4 mm. long.

Known only from Atka Island.

4. *E. virescens* Piper Pacific Rye-grass
E. howellii Scribn. & Merr.

Somewhat tufted; culms 3-12 dm. tall; sheaths smooth; leaves flat, 5-15 mm. wide, minutely scabrous; spike 6-16 cm. long; spikelets few-flowered; glumes strongly nerved, pointed or awn-tipped; lemmas 10-12 mm. long, scabrous toward the sharp-pointed or short-awned apex.

Woods, southeastern Alaska—Calif. (Fig. 187.)

5. *E. canadensis* L. Canada Rye-grass

Culms erect, tufted, smooth, 7-15 dm. tall; leaves 4-20 mm. wide; scabrous; spike 1-3 dm. long, nodding; spikelets 3-5-flowered; glumes narrow, scabrous; lemmas 8-14 mm. long, strongly nerved above, scabrous-hirsute; awns divergently curved, 2-3 cm. long.

Along the Alaska Railroad—Que.—N. Car.—Texas—Ariz.—Calif. Probably introduced in our area. (Fig. 188.)

6. *E. macounii* Vasey. Macoun Rye-grass

Culms densely tufted, erect, slender, 5-10 dm. tall; sheaths smooth; leaves usually scabrous on both sides, 2-5 mm. wide; spike slender, erect or somewhat nodding, 4-12 cm. long; spikelets imbricate, appressed, 1-3-flowered, about 1 cm. long; glumes narrow, scabrous, awned; lemmas 8-10 mm. long, scabrous and somewhat hirsute toward the apex; awns 1-2 cm. long.

Central Alaska—Alta.—Minn.—Nebr.—N. Mex.—Calif. (Fig. 189.)

7. *E. glaucus* Buckley. Western Rye-grass

Culms tufted, often bent at the base, 6-15 dm. tall; leaves more or less

scabrous on both sides, 6–15 mm. wide; spike erect or somewhat nodding, 5–20 cm. long; glumes lanceolate, 8–15 mm. long, with prominent scabrous nerves; lemmas 7–10 mm. long, scabrous toward the apex and with awns 8–20 mm. long.

Southeastern Alaska—Ont.—Mich.—Mo.—N. Mex.—Calif. (Fig. 190.)

8. *E. hirsutus* Presl.

Northern Rye-grass

E. borealis Scribn.

Culms rather weak, 5–14 dm. tall; leaves lax, 4–13 mm. wide, somewhat scabrous beneath, sparsely pilose above; spike loosely flowered, nodding, 10–18 cm. long; spikelets about 15 mm. long; glumes strongly nerved, awned; lemmas long-hirsute on the margins toward the summit, sometimes coarsely pubescent on the back; awn up to 2 cm. long.

Coastal sections. Alaska—Ore. (Fig. 191.)

Pleuropogon sabinii R. Br. is a small grass found in the arctic regions of Canada and Eurasia and may be expected in the most northerly parts of Alaska and the Yukon. It is 15 cm. or less tall; leaves 1–5 cm. long or when growing in water longer; spikelets 2–6, about 1 cm. long on spreading or reflexed pedicels, 5–8-flowered; glumes small, unequal, scarious at the somewhat lacerate tip; lemmas 4–5 mm. long, 7-nerved, the midvein sometimes excurrent as a sharp point; keels of the palea winged on lower half, bearing awnlike appendages near the middle.

PLATE VI

60. *Typha latifolia* L. Inflorescence and flowers.
61. *Sparganium hyperboreum* Laest. Inflorescence, achenes, and perianth scales.
62. *Sparganium angustifolium* Michx. Achenes and scales.
63. *Sparganium minimum* (Hartm.) Fr. Inflorescence, achene, and scale.
64. *Potamogeton natans* L. Leaves.
65. *Potamogeton gramineus* L. Leaves, fruit, and nutlet.
66. *Potamogeton alpinus* Balbis. Leaves and nutlet.
67. *Potamogeton porsildorum* Fern. Leaf, section of leaf, and nutlet.
68. *Potamogeton pusillus* L. Leaf, fruit, and nutlet.
69. *Potamogeton friesii* Rupr. Leaf, tip of leaf, and nutlet.
70. *Potamogeton perfoliatus* L. Leaf and nutlet.
71. *Potamogeton filiformis* Pers. Leaf, fruit, and nutlet.
72. *Potamogeton pectinatus* L. Leaf, fruit, and nutlet.
73. *Ruppia spiralis* L. Leaves, fruit, and nutlet.
74. *Zostera marina* L. Inflorescence, tip of leaf, and nutlet.
75. *Triglochin maritima* L. Leaf, flower, and fruit.
76. *Triglochin palustris* L. Flowers and fruits.
77. *Hierochloe alpina* (Sw.) Roem. & Schult. Spikelet and lemmas.
78. *Hierochloe pauciflora* R. Br. Spikelet.
79. *Hierochloe odorata* (L.) Beauv. Spikelet.

PLATE VI

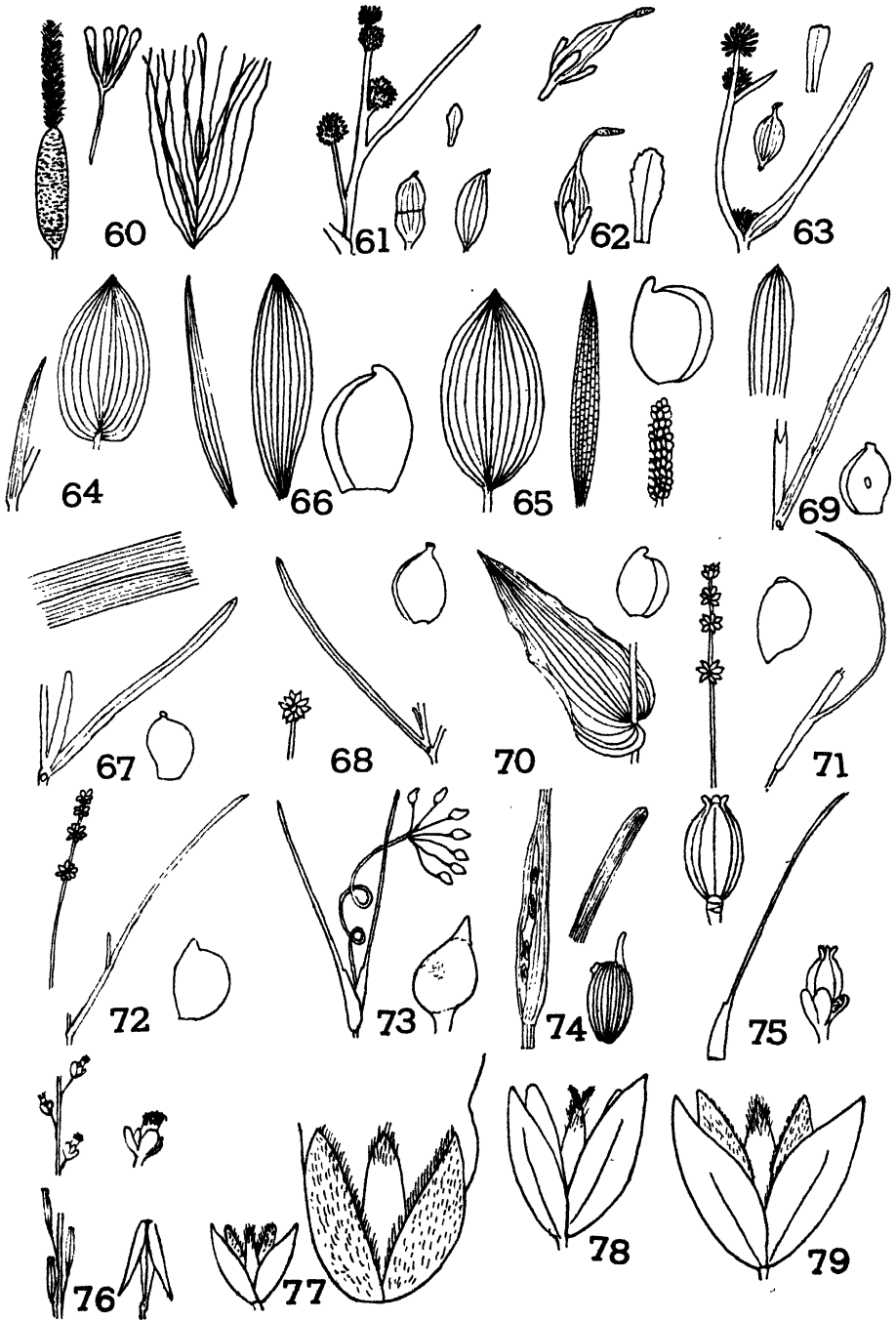


PLATE VII

80. *Phalaris arundinacea* L. Spikelet and lemma.
81. *Anthozanthum odoratum* L. Glumes, sterile lemmas, and fertile lemma.
82. *Stipa columbiana* Macoun. Lemma.
83. *Stipa comata* Trin. Lemma.
84. *Alopecurus pratensis* L. Spikelet.
85. *Alopecurus alpinum* J. E. Sm. Spikelet and lemma.
86. *Alopecurus aequalis* Sobol. Spike, spikelet, and lemma.
87. *Alopecurus geniculatus* L. Spikelet.
88. *Polypogon monspeliensis* (L.) Desf. Spikelet and lemma.
89. *Phleum alpinum* L. Spikelet and floret.
90. *Phleum pratense* L. Spikelet and lemma.
91. *Phippsia algida* (Soland.) R. Br. Glumes and fruiting floret.
92. *Cinna latifolia* (Trev.) Griseb. Spikelet.
93. *Calamagrostis deschampsoides* Trin. Spikelet and floret.
94. *Calamagrostis purpurescens* R. Br. Spikelet and lemma.
95. *Calamagrostis nutkaensis* (Presl) Steud. Spikelet and lemma.
96. *Calamagrostis canadensis* (Michx.) Beauv. Glumes and lemma.
97. *Calamagrostis inexpansa* A. Gray. Glumes and lemma.
98. *Agrostis thurberiana* Hitchc. Glumes and floret.
99. *Agrostis palustris* Huds. Glumes and floret.
100. *Agrostis stolonifera* L. Glumes and floret.
101. *Agrostis exarata* Trin. Glumes and lemma.
102. *Agrostis scabra* Willd. Spikelet and lemma.
103. *Agrostis alaskana* Hult. Glumes and lemma.
104. *Agrostis borealis* Hartm. Glumes and lemma.

PLATE VII

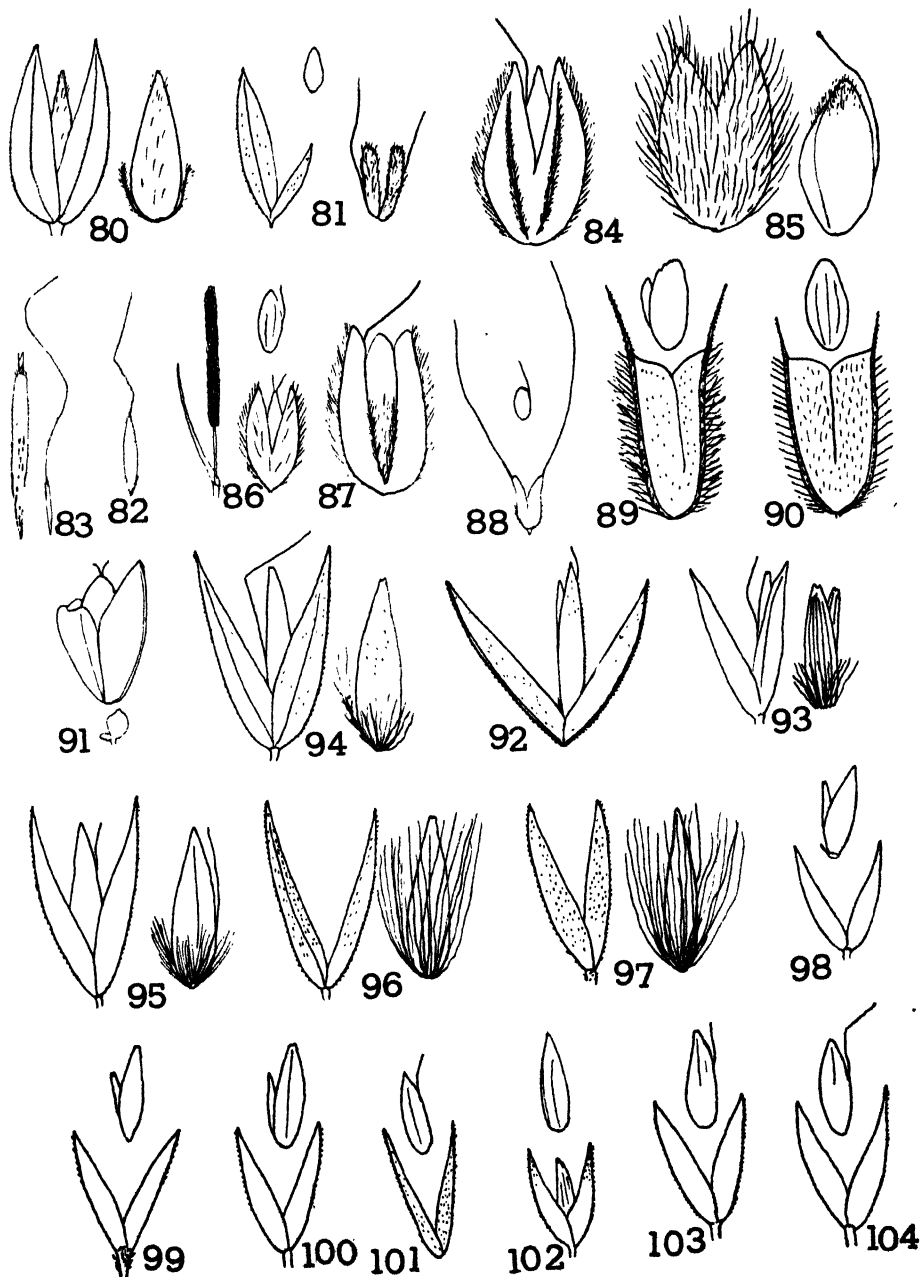


PLATE VIII

105. *Arctagrostis latifolia* (R. Br.) Griseb. Spikelet.
106. *Holcus lanatus* L. Glumes and florets.
107. *Arrhenatherum elatius* (L.) Mert. & Koch. Spikelet.
108. *Sphenopholis intermedia* (Rydb.) Rydb. Spikelet.
109. *Danthonia spicata* (L.) Beauv. Spikelet and lemma.
110. *Avena fatua* L. Spikelet.
111. *Trisetum spicatum* (L.) Richt. Spikelet and lemma.
112. *Trisetum cernuum* Trin. Spikelet.
113. *Trisetum sibiricum* Rupr. Spikelet.
114. *Deschampsia elongata* (Hook.) Munro. Spikelet and lemma.
115. *Deschampsia atropurpurea* (Wahl.) Scheele. Spikelet and lemma.
116. *Deschampsia beringensis* Hult. Spikelet and lemma.
117. *Deschampsia caespitosa* (L.) Beauv. Spikelet and lemma.
118. *Deschampsia holciformis* Presl. Spikelet.
119. *Beckmannia syzigachne* (Steud.) Fern. Spikelet and floret.
120. *Dactylis glomerata* L. Spikelet.
121. *Dupontia fischeri* R. Br. Spikelet and floret.
122. *Schizachne purpurescens* (Torr.) Swallen. Spikelet and lemma.
123. *Colpodium fulvum* (Trin.) Griseb. Spikelet.
124. *Melica subulata* (Griseb.) Scribn. Spikelet and lemma.
125. *Glyceria borealis* (Nash) Batch. Spikelet and lemma.
126. *Glyceria leptostachya* Buckl. Spikelet and lemma.
127. *Glyceria pauciflora* Presl. Spikelet.
128. *Glyceria grandis* S. Wats. Spikelet.
129. *Puccinellia phryganodes* (Trin.) Scribn. & Merr. Spikelet.
130. *Puccinellia hauptiana* Krecz. Spikelet.
131. *Puccinellia grandis* Swallen. Spikelet.
132. *Puccinellia borealis* Swallen. Spikelet.
133. *Puccinellia andersoni* Swallen. Spikelet.
134. *Puccinellia alaskana* Scribn. & Merr. Spikelet.
135. *Puccinellia paupercula* (Holm) Fern. & Weath. Spikelet.
136. *Puccinellia hulteni* Swallen. Spikelet.
137. *Puccinellia pumila* (Vasey) Hitchc. Spikelet.

PLATE VIII

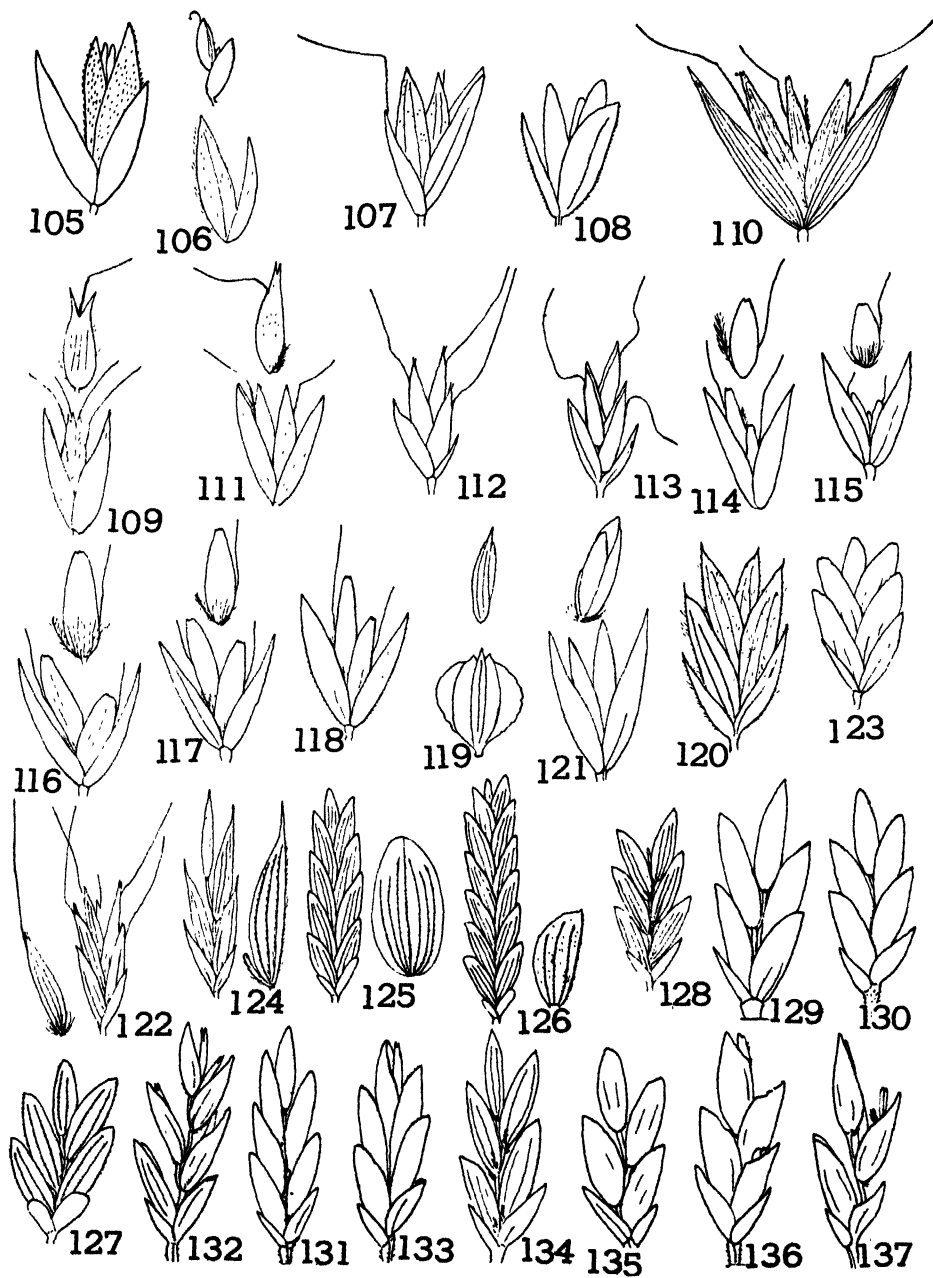


PLATE IX

138. *Puccinellia nutkaensis* (Presl) Fern. & Weath. Glumes and florets.
139. *Poa annua* L. Spikelet and lemma.
140. *Poa compressa* L. Spikelet and lemma.
141. *Poa lanata* Scribn. & Merr. Glumes and lemma.
142. *Poa turneri* Scribn. Floret.
143. *Poa eminens* Presl. Spikelet and lemma.
144. *Poa macrocalyx* Tr. & Mey. Spikelet and lemma.
145. *Poa pratensis* L. Glumes and a lemma.
146. *Poa arctica* R. Br. Lemma.
147. *Poa irrigata* Lindm. Glumes and lemmas.
148. *Poa trivialis* L. Glumes and lemma.
149. *Poa leptocoma* Trin. Glumes and lemma.
150. *Poa nemoralis* L. Glumes and lemma.
151. *Poa palustris* L. Glumes and lemma.
152. *Poa merrilliana* Hitchc. Spikelet and lemma.
153. *Poa alpina* L. Spikelet and lemma.
154. *Poa stenantha* Trin. Glumes and lemma.
155. *Poa glauca* Vahl. Spikelet and lemma.
156. *Poa rupicola* Nash. Glumes and lemma.
157. *Poa hispidula* Vasey. Spikelet and lemma.
158. *Festuca elatior* L. Spikelet.
159. *Festuca subulata* Trin. Spikelet.
160. *Festuca brachyphylla* Schult. Spikelet.
161. *Festuca rubra* L. Spikelet.
162. *Festuca altaica* Trin. Spikelet.
163. *Bromus sitchensis* Trin. Spikelet.
164. *Bromus aleutensis* Trin. Spikelet and lemma.
165. *Bromus marginatus* Nees. Lemma.
166. *Bromus inermis* Leyss. Spikelet.
167. *Bromus pumpellianus* Scribn. Lemma.
168. *Bromus ciliatus* L. Lemma.
169. *Bromus pacificus* Shear. Floret.
170. *Bromus mollis* L. Spikelet.
171. *Bromus tectorum* L. Spikelet.

PLATE IX

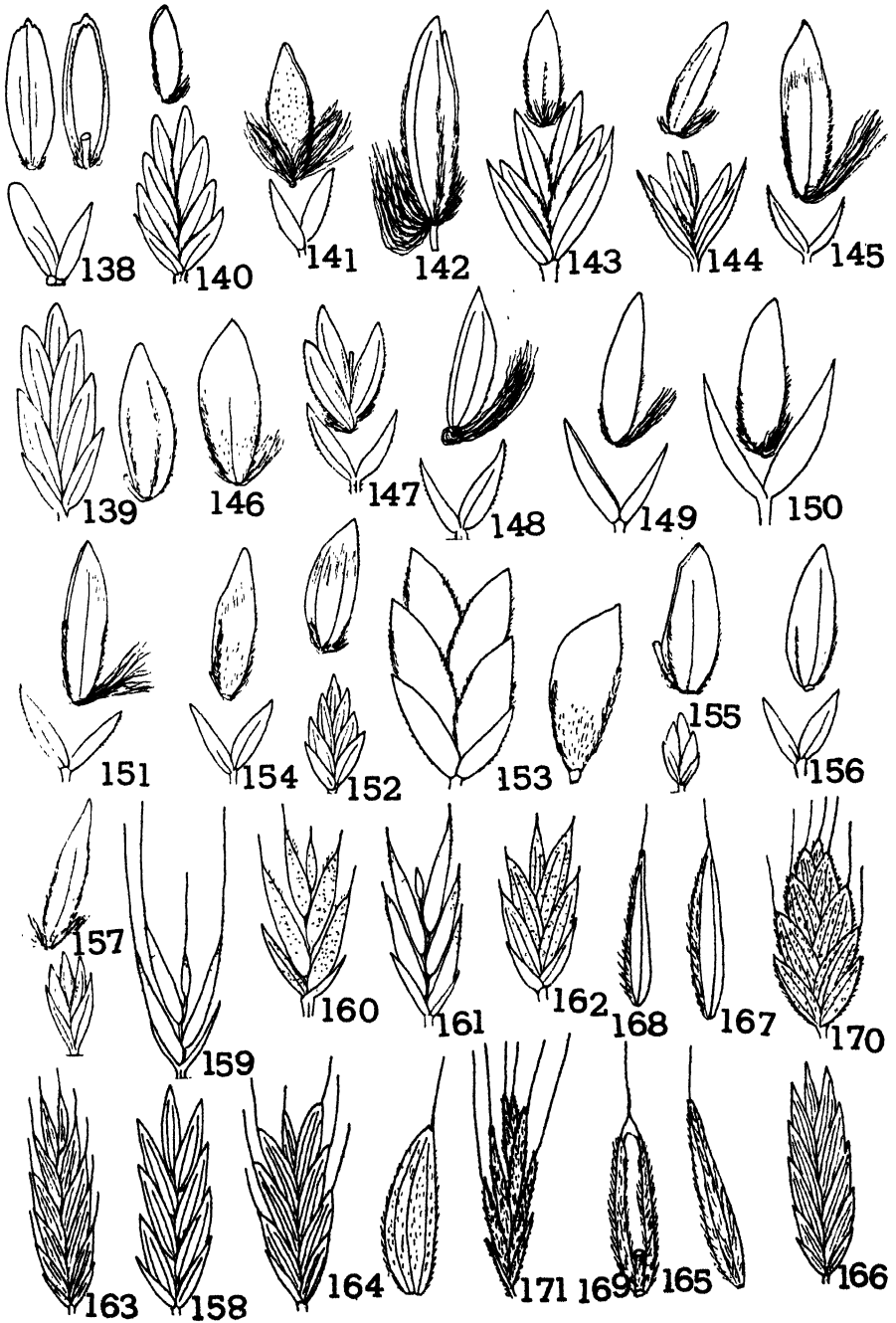
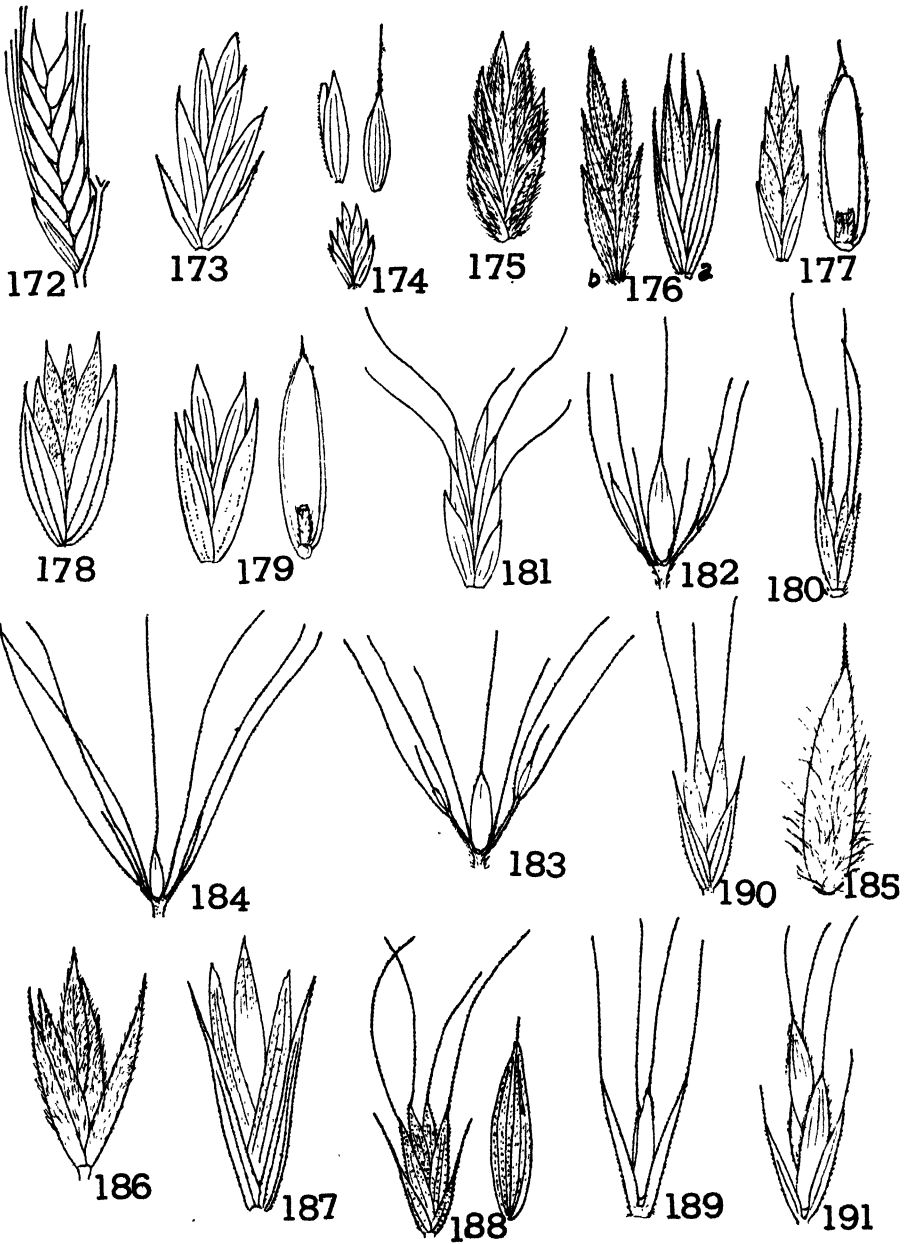


PLATE X

172. *Lolium multiflorum* Lam. Spikelet.
173. *Agropyron smithii* Rydb. Spikelet.
174. *Agropyron repens* (L.) Beauv. Spikelet, floret, and awned lemma.
175. *Agropyron yukonense* Scribn. & Merr. Spikelet.
176. *Agropyron alaskanum* Scribn. & Merr. (a) Spikelet. (b) Var. *arcticum* Hult.
177. *Agropyron sericeum* Hitchc. Spikelet and floret.
178. *Agropyron latiglume* (Scribn. & Sm.) Rydb. Spikelet.
179. *Agropyron trachycaulum* (Link) Hitchc. Spikelet and floret.
180. *Agropyron subsecundum* (Link) Hitchc. Spikelet.
181. *Agropyron spicatum* (Pursh) Scribn. & Sm. Spikelet.
182. *Hordeum brachyantherum* Nevski. Node of 3 spikelets.
183. *Hordeum caespitosum* Scribn. Node of 3 spikelets.
184. *Hordeum jubatum* L. Node of spikelets.
185. *Elymus innovatus* Beal. Lemma.
186. *Elymus mollis* Trin. Spikelet.
187. *Elymus virescens* Piper. Spikelet.
188. *Elymus canadensis* L. Spikelet and lemma.
189. *Elymus macounii* Vasey. Spikelet.
190. *Elymus glaucus* Buckl. Spikelet.
191. *Elymus hirsutus* Presl. Spikelet.

PLATE X



THE INITIATION AND DEVELOPMENT OF FOLIAR AND FLORAL ORGANS IN THE TULIP¹

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The flowering cycles of many bulbous cultivated plants are known primarily through the accumulated practical experience of horticulturists. The presence of a flower bud in the larger bulbs of tulip, narcissus, hyacinth, and other plants has long been recognized. Cultural practices and environmental conditions are known to influence the flower-producing capacity and multiplication of bulbs, and the "running out" or senescence of plantings. The histological details of the initiation and growth of foliar and floral organs have received little attention.

The present report is confined to the tulip, one of the several bulb-, corm-, and tuber-producing ornamentals being studied. The gross morphology of the tulip bulb and the formation of "dropper" bulblets have been described by Arber (1), who also reviewed the early classical studies on bulbous plants. The more recent studies on tulip have dealt with the cytology of the numerous species and varieties (2, 6). The present study was undertaken to determine the approximate date of initiation of foliage leaves and flower primordia under Iowa conditions, to compare varieties having markedly different flowering dates and forcing properties, and to compare the structural maturity of bulbs from different commercial sources. A preliminary study was made of the relationship of bulb size to bud formation. The revival of interest in the cyto-histology of the shoot apex, reviewed recently by Foster (5), has led to the first detailed study of this feature of the tulip.

MATERIALS AND METHODS

Tulip bulbs were obtained through commercial channels in October, 1939. These bulbs were represented as having been grown in Holland, and were of the size classification that is usually expected to bloom during the season following planting. Varieties were selected to represent early-blooming as well as relatively late-blooming classes. Sample bulbs of each variety were dissected to ascertain the presence of a flower bud. Plantings of bulbs were made in the Herbaceous Gardens in October. Samples were dug in November and at intervals during the following growing season.

Bulbs grown in the Tacoma, Washington, district were obtained in 1941. A sample of each variety was examined on receipt; the remainder were stored under good curing conditions and planted in November.

¹ Journal paper No. J-1188 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 687.

Samples were taken at intervals during storage and during the subsequent growing season.

Through the courtesy of Prof. E. C. Volz, bulbs were also obtained from well-established plantings in the gardens of the Horticulture Department of Iowa State College, Ames, Iowa.

Acetocarmine smears were made to determine the stage of development in the larger anthers. Diagnoses of small anthers and of ovaries were made from microtome sections of entire flower buds. The buds were dissected out of the bulb, killed in chrome-acetic formalin, CRAF No. 2 (8), embedded in paraffin, and sectioned.

OBSERVATIONS

FLOWERING CYCLES OF EUROPEAN AND AMERICAN BULBS

Seven varieties of Holland-grown bulbs of large commercial size were examined on October 20. These bulbs had been subjected to the shipping and storage conditions of the trade and were in the condition in which they appear in the retail outlets. A fifteen-bulb sample of each variety was dissected. Every bulb was found to contain a well-developed flower bud, all organs of which were macroscopically recognizable. Microscopic study of sections showed that in all cases the ovules were short, straight primordia, with no trace of integuments, and with indistinguishable, or only slightly enlarged sporocytes. This striking uniformity of the condition of the ovules in the earliest and latest varieties was in contrast with differences in the cytological condition of the anthers. The following tabulation of seven varieties is arranged in order of relative maturity of pollen on October 20. The class is given in parentheses.

Bleu Aimable (Darwin), microsporocytes in meiotic prophase.

Wisconsin (Triumph), Clara Butt (Darwin), first meiotic division to early quartets.

Wm. Pitt and Pride of Haarlem (Darwin), quartets.

Kaisererkroon (Early Single), late quartets to microspores.

Mendel, mixed colors (Mendel), microspores.

The early tulips, Kaisererkroon and the Mendels, clearly showed a more advanced condition of pollen. Wisconsin, an early-blooming Triumph, was in approximately the same condition as the much later-blooming Darwin, Clara Butt. The good forcer, Wm. Pitt, and the non-forcer, Pride of Haarlem, had attained identical morphological maturity.

Collections were made on November 20, after several severe frosts had occurred, and the bulbs had probably become dormant. All of the above varieties had attained essentially the same stage of pollen development. Anthers contained gradations from late quartets to immature, uni-nucleate pollen (microspores). The initial precocity of floral development of the early varieties had been overcome by the time of dormancy.

The morphogenesis and cytology of the ovary and ovules were not within the scope of the present study, therefore, collections were not made until after anthesis. The first collection was made on July 1, when

the vegetative leaves of tulips are still in good condition in this region. The stem apex, from which next year's flower will develop, is deeply buried near the base of the bulb. The structure of this stem tip can be reconstructed readily from transverse and longitudinal microtome sections. No floral primordia were found in any of the foregoing varieties. The initiation and rapid enlargement of successive leaf primordia were taking place. Numerous mitotic figures were evident in the promeristem and in the leaf primordia.

On August 1, Clara Butt and Pride of Haarlem had completed the initiation of vegetative leaves, but no floral primordia were evident (Fig. 1). The other varieties of this planting had perianth and stamen primordia, listed here in the order of diminishing size of floral organs: Wisconsin, Kaiserkroon, Mendel, Wm. Pitt, and Bleu Aimable. At this date, the stage of floral development was in approximately the same order as earliness of blooming in the varieties.



FIG. 1. Median longi-section of apex of vegetative bud of Pride of Haarlem tulip, July 15. 60 \times .

The stage of pollen development on September 20 is shown in the following tabulation.

Kaiserkroon, Pride of Haarlem, Bleu Aimable, and Clara Butt, early prophase of microsporocytes.

Wm. Pitt, strepsitene to diakinesis in the microsporocytes.

Mendel and Wisconsin, pollen quartets.

Although the early classes, Mendel and Wisconsin, were well advanced in pollen development, it should be noted that the early single variety, Kaiserkroon, was in the same stage as most of the Darwins. The good forcer, Wm. Pitt, was somewhat more advanced than the non-forcer, Pride

of Haarlem. The degree of ovule development was uniform in all varieties, and approximately at the same stage as on October 20 of the previous year. Megasporocytes had not undergone enlargement in any of the varieties.

These observations show that the foliage leaves that emerge with the flower are probably initiated by July 1. Ovules develop very slowly from the time of initiation until dormancy, the megasporocyte becoming barely distinguishable. Anthers develop rapidly, meiosis takes place, and an advanced condition is attained by the time of dormancy, the dormant anthers containing loosely joined quartets or separated microspores.

Further studies had been planned to compare the structural maturity of Dutch and American bulbs, and to ascertain the extent of year-to-year



FIG. 2. Pride of Haarlem, initiating stamen primordia on July 20. 48 \times .

variation in foreign and domestic bulbs. The destruction of the bulb industry in the Netherlands has closed that source of stock. Subsequent studies have been limited to further comparisons of classes, and to the cyto-histology of the flower-producing stem apex. Having shown that the buds of a wide range of varieties have essentially the same morphological conditions at dormancy, only two varieties were used subsequently. An "early single" variety, Spring Glory, and a considerably later-blooming Darwin, Pride of Haarlem, were obtained from Tacoma, Washington.

When received in Ames, Iowa, on June 15, 1941, bulbs of both varieties were in the vegetative condition. Active cell division was evident in the stem apex and in the leaf primordia, particularly along the marginal meristems of the latter, as in figs. 1, 5-7. The bulbs were put into storage on curing trays until planting time, and samples were taken on the indicated dates.

Perianth primordia became evident on Spring Glory by July 10,

whereas *Pride of Haarlem* was still in the vegetative condition on this date. On July 20, *Pride of Haarlem* was just initiating stamen primordia (Figs. 2, 8-9). All fifteen bulbs of the sample had recognizable stamen primordia. The broad primordia extended laterally, not emerging above the level of the stem apex. Measured from the axil to the tip, the greatest stamen length found was 90 μ , and the largest perianth primordia were 180 μ in length. On the same date, *Spring Glory* was distinctly more advanced in floral development. The anthers had attained a maximum length of 360 μ , protruding well above the apex of the axis. In transverse section the anthers had become deeply four-lobed, but the archesporia had not yet become delimited. Active cell division was evident throughout the anthers especially in the central regions of each lobe. The three-angled form of

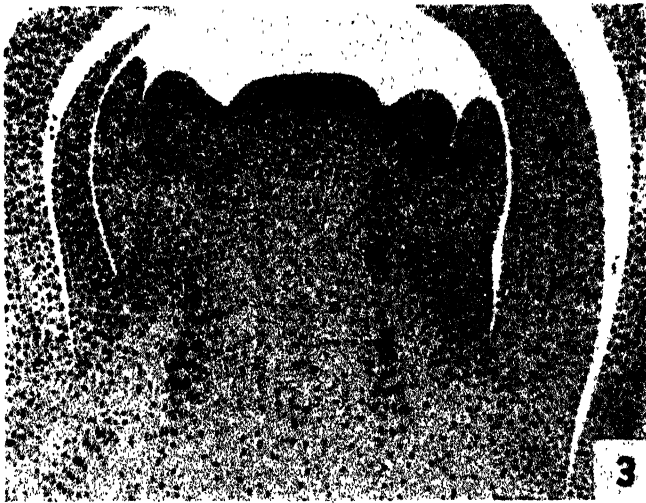


FIG. 3. *Pride of Haarlem*, enlarged stamen primordia on July 26. 48 \times .

the ovary of *Spring Glory* was evident by July 20. *Pride of Haarlem* had developed a triangular ovary and perceptibly four-lobed stamens by August 1 (Fig. 4).

Collections were made from the above plantings during the next growing season, the first season for these Washington bulbs growing under Iowa conditions. On July 15, both *Spring Glory* and *Pride of Haarlem* were in the vegetative condition. Perianth primordia were evident in *Spring Glory* on July 20, and in *Pride of Haarlem* on August 5. On November 10, both varieties had microspores.

The foregoing observations on American-grown bulbs, supporting the evidence obtained from Dutch bulbs, show that the early-blooming tulips initiate floral organs earlier than do the late-blooming varieties, but that by late autumn, both classes attain essentially the same morphological condition. A given variety develops its flower bud during approximately the same time of the season, regardless of the source of the bulbs. Differ-

ences of a week to 10 days in the date of floral initiation in successive years may well be ascribed to environmental fluctuations. The slightly earlier formation of flower primordia in the original Washington bulbs is ascribed to the fact that they were dug and cured before the customary digging time, and the early destruction of the tops induced differentiation of flower primordia.

The comparison of American and foreign stock has lost all importance since these studies were begun. Emphasis should now be placed on comparative developmental studies of stock from the several American pro-



FIG. 4. Trans-section of flower bud of Pride of Haarlem, August 1. 48 X.

ducing regions, in relation to dates of harvesting, conditions of curing and storage, and other cultural practices.

RELATION OF BULB SIZE TO FLOWER PRODUCTION

The ability of a bulb to produce a flower has long been known to be associated with bulb size. The larger commercial sizes are generally "guaranteed" to bloom in the spring following planting. Small bulbs may either fail to bloom, or may produce flowers of inferior size. A study was undertaken to determine the relationship between bulb weight and the anatomical development of the flower bud. Ten varieties that had become established in the Horticultural Gardens were used. Sample bulbs were taken from the curing trays on December 1, weighed and split longitudinally to ascertain whether a flower bud was present. Bulbs in which a flower bud was not visible to the unaided eye were dissected, and the

apical region of the stem was removed and processed for sectioning in paraffin.

All bulbs in which a flower bud was not visible to the unaided eye were found to be in the vegetative condition. The stem apices bore foliage leaf primordia and young leaves, resembling Figure 1. Floral primordia were not found at the end of the growing season. The conclusion can be drawn that a given bulb is either strictly vegetative, or it has a well-developed flower bud, containing nearly mature pollen and the rudiments of ovules.

The minimum bulb weight necessary for the formation of flower bud was investigated, using the foregoing material. Bulbs were classified as falling within designated weight limits; below 4 g., 4-5 g., 5-6 g., etc. Having found that if floral organs are present at all, they are readily visible macroscopically in a bulb cut lengthwise, this method of examination was used. The following list gives the minimum weight range in which a flower bud was present.

Telescopium (Triumph class)	5-6 g.	Vesta (Cottage)5-6 g.
Columbia	"	Moon5-6 g.
Lucifer (Breeder)5-6 g.	Yellow Giant (Darwin)4-5 g.
Rembrandt7-8 g.	Wm. Pitt	"5-6 g.
Parrot6-7 g.	Bartigon	"5-6 g.

A value between 5 and 6 grams seems to represent an average minimum size of bulb in which a flower bud is produced. This size is well below the size that is customarily sold to gardeners. The presence of a flower bud in these small bulbs apparently does not insure the production of a flower. It is probable that the flower buds of undersized bulbs fail to emerge, or that such bulbs give rise to the "blasted" buds that emerge but fail to develop into acceptable flowers. Experiments are in progress to determine the correlation between bulb weight, dimensions, and the presence of a bud, and the emergence of a flower of desirable size and quality.

These studies have some bearing on the "running out" or senescence of plantings of tulips. In regions having hot, dry weather during the early part of the growing season, the formation of next year's foliage leaves may well be stopped early in the season. The photosynthetic activity of the current season's leaves is certainly inhibited. Flower buds, which are known to be initiated early, probably do not emerge as full-sized flowers. The behavior of several varieties that seem to thrive under such adverse conditions is now being studied.

THE CYTO-HISTOLOGY OF THE SHOOT APEX

The vegetative apex of the principal axis in the tulip bulb is deeply buried, situated just above the disc-like basal stem, and is covered by the numerous fleshy scale leaves. The entire "terminal bud" is 2-3 mm. long and 1-2 mm. broad. The stem apex is a short, broad, smooth dome, 100-125 μ high and 300-375 μ broad. In median section, the outline is more or less semi-circular (Figs. 1, 5). The tunica of the vegetative apex consists

of a single layer of cells. This is particularly clear-cut in sections having numerous mitotic figures (Fig. 6). In sections having few mitotic figures, the orientation of obvious sister-cells suggests that during periods of slow mitotic activity, at least three layers undergo some anticlinal cell division.

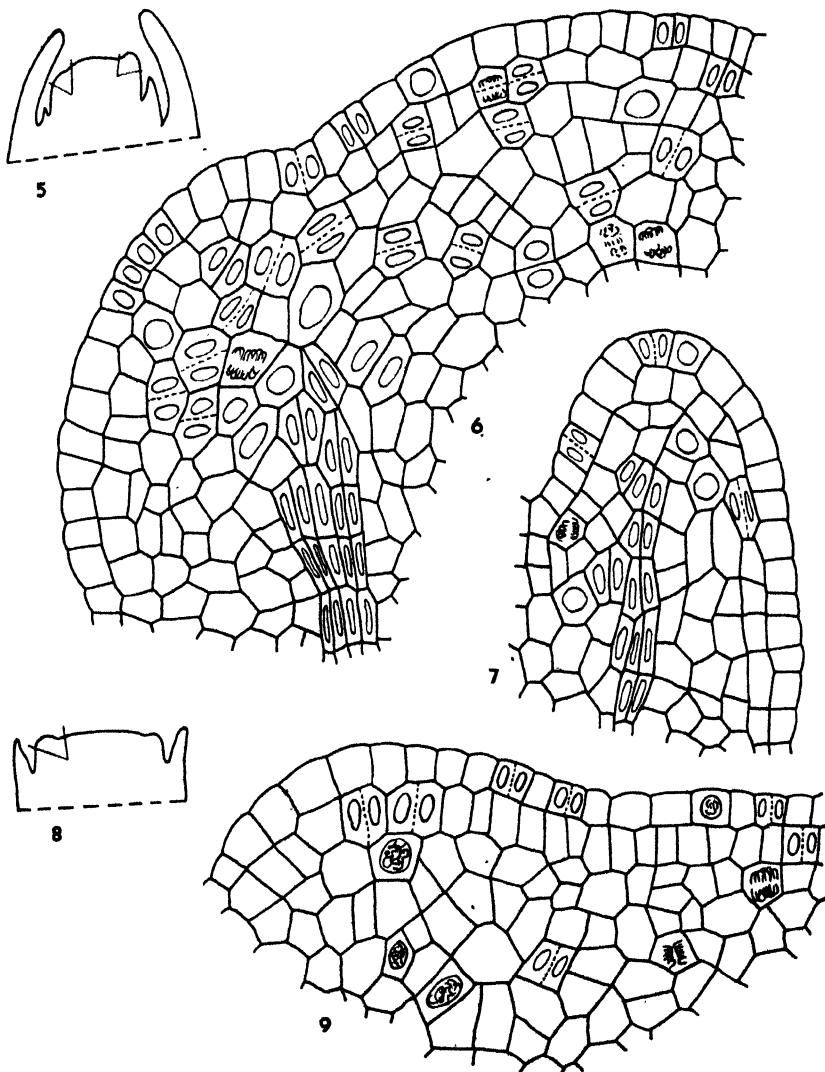


FIG. 5. Median longi-section of vegetative shoot apex of Pride of Haarlem, July 15. 25 \times .

FIG. 6. Detail of youngest leaf primordium and procambium strand from Figure 5. 250 \times .

FIG. 7. Detail of second leaf primordium and procambium strand from Figure 5. 250 \times .

FIG. 8. Median longi-section of shoot apex of Pride of Haarlem, initiating stamen primordia, July 20. 25 \times .

FIG. 9. Detail of stamen primordium from Figure 8. 250 \times .

Leaf primordia are initiated by activity in the first layer of the corpus, soon involving a zone of cells to a depth of three to four cells (Fig. 6). A comparatively massive region is thus responsible for the enlargement of the leaf primordium. The tunica of the primordium and young leaf retains its identity as a single layer of cells (Fig. 7).

The primordia of the perianth and the stamens arise in the same manner as leaf primordia, and enlarge by cell division in the corpus. The apex remains dome-shaped during the initiation of the perianth and stamens, and the tunica of the apex and of the primordia is maintained as one layer of cells (Fig. 9).

Soon after the stamens have elongated above the level of the apex, a change occurs in the shape and cellular organization of the apex. The vertical sectional shape changes from semicircular to a nearly flat-topped, square outline (Fig. 3). Whereas the tunica of the vegetative apex consists of one layer of cells, that of the floral apex becomes two-layered. Cell division is strictly anticlinal in the outer layer, and predominantly so in the second layer. The gradual shift from the one-layered to the two-layered condition becomes evident soon after the stamen primordia are laid down. A change of organization at this period of floral development was noted in *Amygdalus* by Brooks (3). The subsequent development of the carpels has not been studied. The condition of the ovules in late summer and during dormancy has been described earlier in this paper.

The large cells and large, deeply stainable nuclei of the promeristem of the tulip make this a favorable subject for the study of procambium strands. In serial longitudinal sections, the prominent strands in the second or third node can be traced upward until they become more obscure in the zone of leaf initiation. Strands can be recognized in the youngest distinguishable leaf primordium. The strands are usually oblique with respect to the axis of the primordium, but serial reconstructions show that the strand is continuous from the principal axis into the leaf initial. The procambial cells are progressively shorter acropetally, until the strand can be distinguished only by the tendency of the planes of division to be anticlinal to the curve of the primordium (Fig. 6). Strands have been traced into the leaf primordium to the fourth layer of the corpus. There is evidence of strand formation in close proximity to the zone of leaf initiation, before the potential leaf primordium protrudes perceptibly out of the apical dome. This is similar to the condition described in *Linum* by Esau (4). Whether the inception of a strand is the precursor or the consequence of leaf initiation is still an open question.

SUMMARY

A study was made of the seasonal sequence and histology of leaf and flower initiation in the tulip.

Tulip bulbs of commercial size contain a well-developed flower bud when the bulbs become available in the retail trade. In the seventeen varieties examined, anthers contained microspores, whereas megasporocytes had undergone little or no enlargement by November.

Bulbs weighing as little as 5 g. may contain a flower bud, having, at dormancy, the maximum structural development described above.

No partially developed flower buds were found during dormancy. Bulbs either contain only leaves, or a flower bud as described above.

Under garden conditions in Iowa, early varieties initiate floral primordia between July 10 and 15, late varieties between July 15 and 20. All varieties examined attain the same degree of structural development by late autumn.

The vegetative promeristem has a tunica of one layer of cells. Leaf primordia arise by accelerated cell division in the first layer of the corpus, the activity soon extending to at least four layers of the corpus. Perianth members and stamens arise in the same manner as foliage leaves.

After the initiation of stamens, a gradual transition occurs from a one-layered to a two-layered tunica in the zone of carpel initiation.

Procambial cells can be recognized in the fourth layer of corpus cells from the tip of the youngest primordium. There are indications of procambial initiation prior to the emergence of a leaf primordium.

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THE STROPHIOLE AND OTHER SEED STRUCTURES ASSOCIATED WITH HARDNESS IN *MELILOTUS ALBA* L. AND *M. OFFICINALIS* WILLD.

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"Hardness" in sweet clover seeds, a condition that prevents the seeds from imbibing water and germinating under favorable germinating conditions, is of much agronomic importance for which reason it has already received considerable study. As a rule more than 90 per cent of the well-ripened normal seeds are hard at the time of harvest. The abrasive and other mutilating effects of the machinery in general use in harvesting the seeds reduce considerably the percentage of hardness, but generally the reduction is not more than 30 or 40 per cent.

It is well known that hardness is due to the impermeability of the seed coat. In natural seedings the seed coat requires the weathering of one or more seasons to effect permeability, while in agronomic practice permeability is induced by artificial scarification.

The seed coat (text figure 1) is rather complex, consisting of a number of structures, of which one or more may be the cause of hardness. Hardness is quite generally ascribed to the outermost cell layer of the seed coat, known as the malpighian or palisade layer. This layer consists of several closely related structures which, beginning with the outermost, are known as the cuticle, cuticularized layer, domes, light line, and the inner cell portions which contain the cell lumina and protoplasmic contents. These structures, especially the cuticle, cuticularized layer, domes, and light line are distinguished under the microscope, chiefly by differences in refraction. Interior to the malpighian layer is the osteosclerid layer, one cell in thickness with lateral walls irregularly thickened in ridges, and contributing to the strength, rigidity and the insulating properties of the seed coat. The several layers of less modified cells which form the inner portion of the seed coat function in the manufacture and storage of food during the development of the seed, and add to the protective function of the seed coat in mature seeds (fig. 1).

Although investigators are almost unanimous in locating the cause of hardness in the outer portion of the malpighian layer, there is still a question as to the particular structures involved. Rees (7) and Hamly (4) have implicated both the cuticularized layer and domes. They concluded that the domes are impermeable and are held together so tightly at their bases by the cuticularized layer that they form a continuous impermeable layer. Coe and Martin (3) and Stevenson (9) attributed hardness to the light line.

Hamly's (4) investigations led him to introduce an innovation into the problem, namely, that hardness or softness depends upon whether or

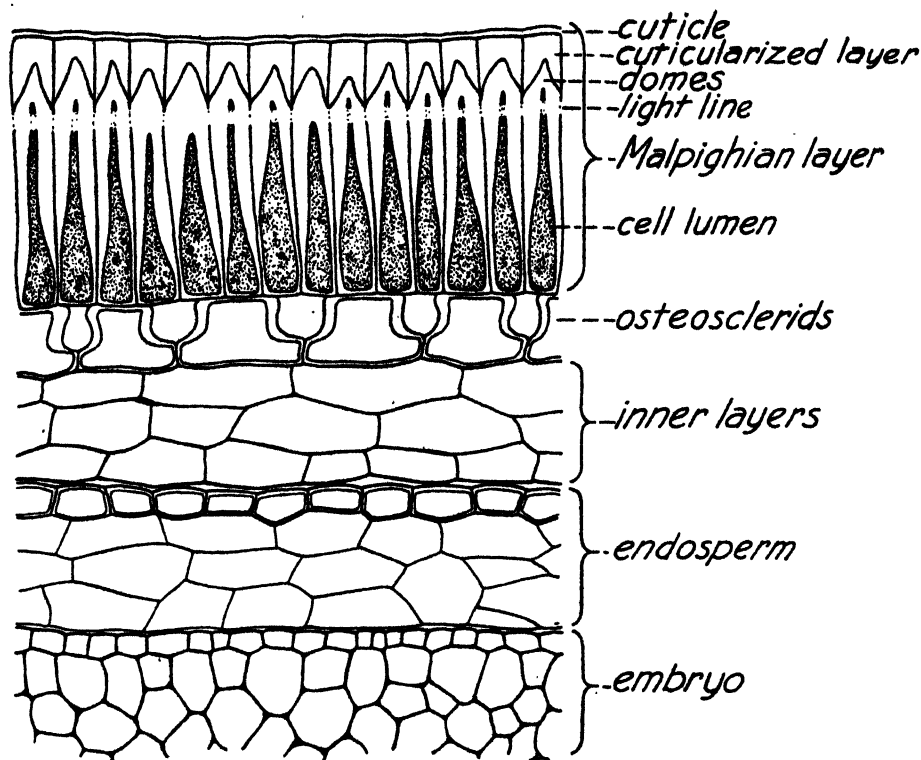


FIG. 1. A drawing, somewhat diagrammatic, of the structures of a sweet clover seed as seen in a vertical section of the seed.

not the strophiole of the seed is closed or open to the entrance of water. The strophiole, a small elongated depression surrounded by a raised border, is a hilum structure with its location at the side opposite the micropyle. This discovery Hamly made by immersing the seeds in a solution of osmic acid and observing the entrance of water as indicated by the blackening of tissues invaded by the acid. Shortly after soft seeds were immersed in the acid, their strophioles were observed to blacken, and the blackening was soon followed by a swelling of the seeds. Hard seeds, in contrast to soft seeds, showed no blackening of strophioles or evidence of swelling after days of immersion in the acid. Scarifying and impacting were reported to be effective in softening hard seeds insofar as they opened the strophiole. This relation of strophiole to hardness, Hamly observed in several leguminous species and thought it likely a feature characteristic in general of hard leguminous seeds.

The findings of more recent investigations as those by Bredeman (2), Behrens (1), and Stevenson (9) are considerably at variance with those of Hamly. Bredeman, after applying Hamly's method to a number of leguminous seeds including those of alfalfa, hairy vetch, and red, white,

and crimson clover, reported that many soft seeds did not blacken and some hard seeds blackened at the strophiole. Behrens (1) reported similarly in regard to a number of leguminous seeds, including those of sweet clover, red clover, and alfalfa. Stevenson's (9) investigations of the absorption of water by sweet clover seeds led him to conclude that water absorption is not limited to any particular area in either naturally hard and soft or artificially softened seeds. Kuhn (6) observed that in the germination of lupine seeds, the first rift in the seed coat occurred at the strophiole but not till after the seed had absorbed some water and had begun to swell.

The endosperm of sweet clover and other leguminous seeds, although regarded as absent or insignificant by the older botanists, may possibly play a very important role in longevity and germination. Harz (5) describes the endosperm of *Melilotus alba* and *M. officinalis* as consisting of an outer layer of gelatinous cells with thick walls and of one or more gelatinous inner layers of thin-walled cells, which are omitted over the radicle. He reported that the elements of the endosperm are chiefly those of the gelatinous materials, no starch and very little protein being present. Schulz (8) and Terras (10) held that the gelatinous endosperm in sweet clover opens the seed coat and facilitates the liberation of the cotyledons from the seed coat.

THE INVESTIGATION AS TO OBJECT, MATERIAL, AND METHODS

The diversity of reports concerning the strophiole in relation to hardness in sweet clover seeds prompted this investigation. The investigations were confined to the biennial and annual varieties of white sweet clover, and the biennial variety of the yellow species.

The samples of seeds used represented various types of treatment; they included samples of seeds freshly picked from plants and hulled by hand, samples of seeds proven hard by germinating tests, samples of hard seeds softened outdoors by weathering, samples of hard seeds scarified by hand between sandpapers, and samples of seed scarified by commercial scarifiers. The history of all samples was well known.

The information concerning the development and structure of the strophiole and other seed structures related to hardness was obtained from sections of seeds in various stages of development. The seeds were the product of bagged and hand pollinated flowers. The seeds were collected at intervals of 2 days over a period of 20 days following pollination. The killing and fixing by Bouin's or alcohol-formalin-acetic acid solutions followed by the paraffin method of processing, gave good results. Various stains as haematoxylin, safranin, and fast green, in the usual combinations, were satisfactory.

A 1 per cent aqueous solution of osmic acid into which the seeds were immersed was used to locate on the seed coats the places of initial water absorption. A blackening at the strophiole or any other region followed by a swelling and spreading of the acid effects was regarded as decisive evidence that water was entering at that place.

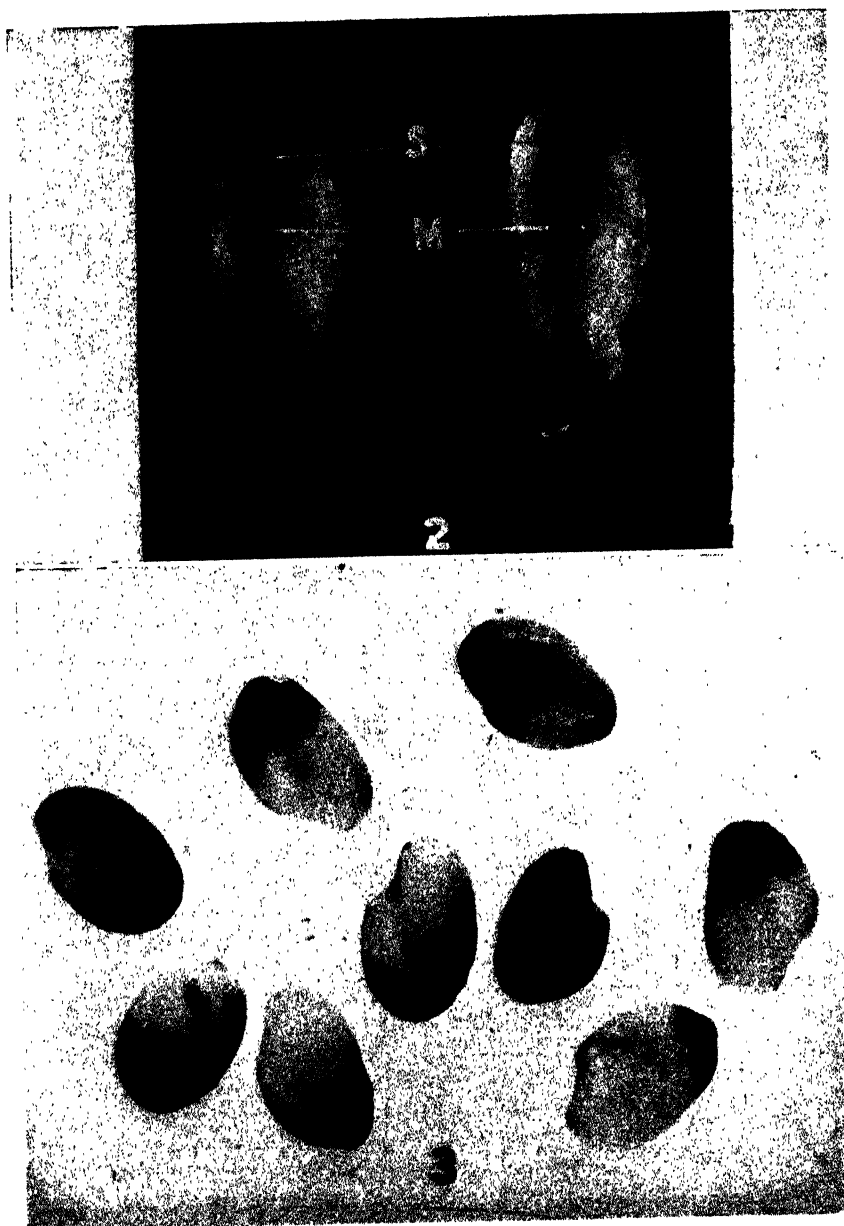


FIG. 2. Surface view of a hard seed (left) and soft seed (right) of sweet clover, showing the strophylar (S) and micropylar (M) areas of each and their difference in reaction to osmic acid, the blackening at the strophylar being distinctive of the soft seeds.

FIG. 3. Hard sweet clover seeds after being stored outdoors for three years. They blackened at the strophylar and one or more other places when exposed to osmic acid.

The blackening by osmic acid results from the action of the acid chiefly on the organic contents of the cells, and principally of the malpighian cells. The contents of the other seed coat layers and endosperm usually darken more or less but not so strikingly as those of the malpighian layer. The initial blackening of the strophiole, which is a mere black dot when first noticeable, most likely results from the reaction of the acid in the vascular bundle entering the seed.

The structure and relational features of the endosperm were ascertained in part from the prepared slides of the processed seeds and in part from a study of green mature seeds which were dissected into their components. Fully developed seeds still retaining their growth moisture content, are rather easily dissected without any material distortion of structures, if the manipulation is done under water where the seed coat, endosperm, and embryo are easily disengaged. The turgid state of these structures and the ease with which they can be oriented in water, greatly facilitate the study of their structure and relationship.

TABLE 1

A SUMMARY OF THE REACTIONS OF THE SWEET CLOVER SEEDS OF THREE SEASONS TO A 1-PER CENT OSMIC ACID SOLUTION

The Separations and Their Treatment	Percentage of Seeds Reacting to Osmic Acid After 48 Hours and Kind of Reaction				
	Position of Initial Blackening			No Blackening Percent- age	Swelling in the Acid Percent- age
	Only at the Strophiole Percent- age	At Strophiole and Elsewhere Percent- age	At Places Other Than Strophiole Percent- age		
Fresh hand-harvested seeds . . .	6	2	1	91	9
Fresh hand-harvested seeds after 2 months in the germi- nator	4	0	0	96	4
Hard seeds taken from germi- nator and rubbed between sandpapers	42	28	16	14	86
Seeds not reacting to the acid, scarified and returned to the acid	48	24	6	22	78
Hard seeds softened outdoors by the weather	78	6	0	16	84
Hard seeds with strophiolar area lightly rubbed with sandpaper	83	17	83
Scarified seed from commer- cial seed houses	24	38	16	22	78

RESULTS

STROPHIOLE IN RELATION TO WATER ABSORPTION

In every batch of sweet clover seeds, even in commercially cleaned samples, there is a variable number of imperfect seeds, which chiefly for lack of time to mature or through injuries in threshing, are not normal in behavior. They are prevailingly soft and generally blacken in a few minutes in osmic acid, the blackening beginning at various places on the seed. By carefully assorting the seeds with the aid of a microscope, it was possible to reduce the imperfect seeds in the separations investigated to almost a negligible number.

The summarized data in Table 1 show conclusively that the strophliar region was the place of initial water absorption in practically all normally developed fresh seeds. Almost 100 per cent of the hard sweet clover seeds showed no reaction to the acid previous to softening treatments (fig. 2). After hard seeds were softened by weathering or scarification, the first evidence of water absorption was prevailingly at the strophiole. In many of the hard seed separations that were stored outdoors until softened by the weather, water absorption was limited 100 per cent to those seeds that blackened at the strophiole, and likewise, to the same extent, the seeds in the separation that remained hard showed an absence of blackening at the strophiole and elsewhere. The strophliar blackening, invariably accompanied by swelling, spreads rapidly throughout the seed coat, usually reaching all parts of the coat in a few minutes (fig. 2).

Hard seeds were easily softened by stroking their strophliar areas with sandpaper or similar abrasive material. Only light scarification of the strophliar region was required to open it to the entrance of water. The obstruction to the entrance of water at the strophiole is evidently on or very near the surface and of rather delicate thickness, features suggesting an abscission layer.

In case of soft seeds in storage, especially when stored under variable

FIG. 4. Showing various stages in the transformation of the outer integument into the seed coat.

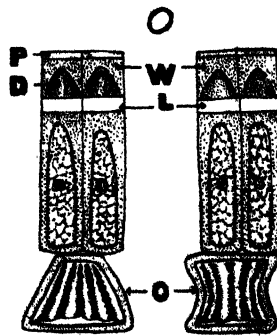
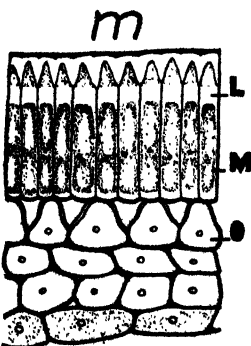
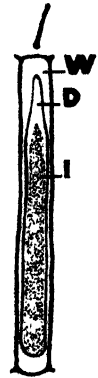
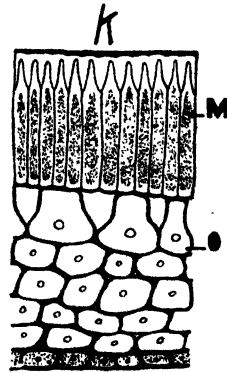
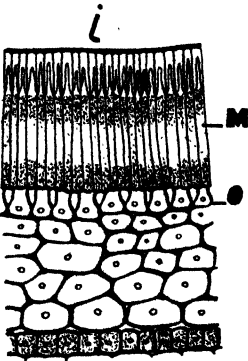
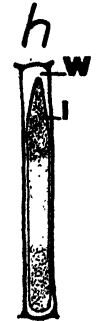
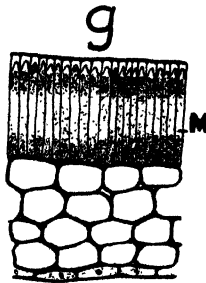
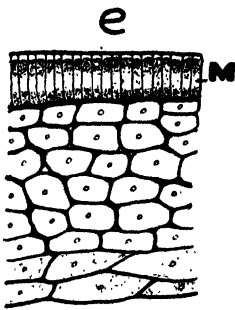
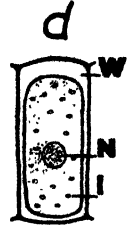
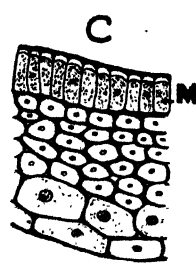
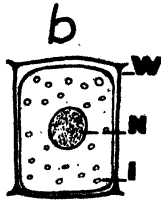
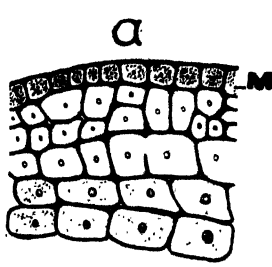
The illustrations *a* and *b* show the shape and other characters of the epidermal cells (M) of the outer integument at the time of fertilization and a day or so following. In *b* some secondary thickening (W) of the outer cell wall has occurred.

The illustrations *c-o* show the rapid elongation, multiplication, and thickening of the outer walls of the epidermal cells. In *c* and *d*, stages about 72 hours after pollination, the epidermal cells are noticeably elongated and outer walls considerably thickened. Stages *e-h* covering 4 to 10 days after pollination, show the epidermal cells decidedly elongated and the secondary thickening (W) of their outer walls quite pronounced.

Stages *i* and *l* show the early development of the domes (D) and the initial stages in the formation of the osteosclerids (O) from the subepidermal layer.

Stages *m-o*, which occur 14 to 20 days after pollination, show the domes (D), light line (L), and cuticularized layer (W) as distinctive structures.

Stage *o* shows the mature stage of the malpighian and osteosclerid cells. The primary cell wall (P), cuticularized layer (W), domes (D), light line (L), and interior portions of the cells containing the protoplasm (I) are now well-defined structures of the malpighian cells; and the osteosclerid cells (O), which are of the two shapes represented, have their ridgelike thickenings that are characteristic of mature seed coats.



weather conditions similar to those outdoors, the seed coats rapidly deteriorate and open at various places to water absorption. This is true also for hard seeds after they are softened by weathering or artificially. Small samples of hard seeds, which were stored in an open garage, were 91 per cent soft after 1 year, and the osmic acid test showed that water absorption was restricted in nearly all softened seeds to the strophiole. After 3 years many of the seeds were dead, and the seed coats of the majority were open to water absorption at various places other than the strophiole (fig. 3).

STRUCTURAL DEVELOPMENT OF SEED COAT

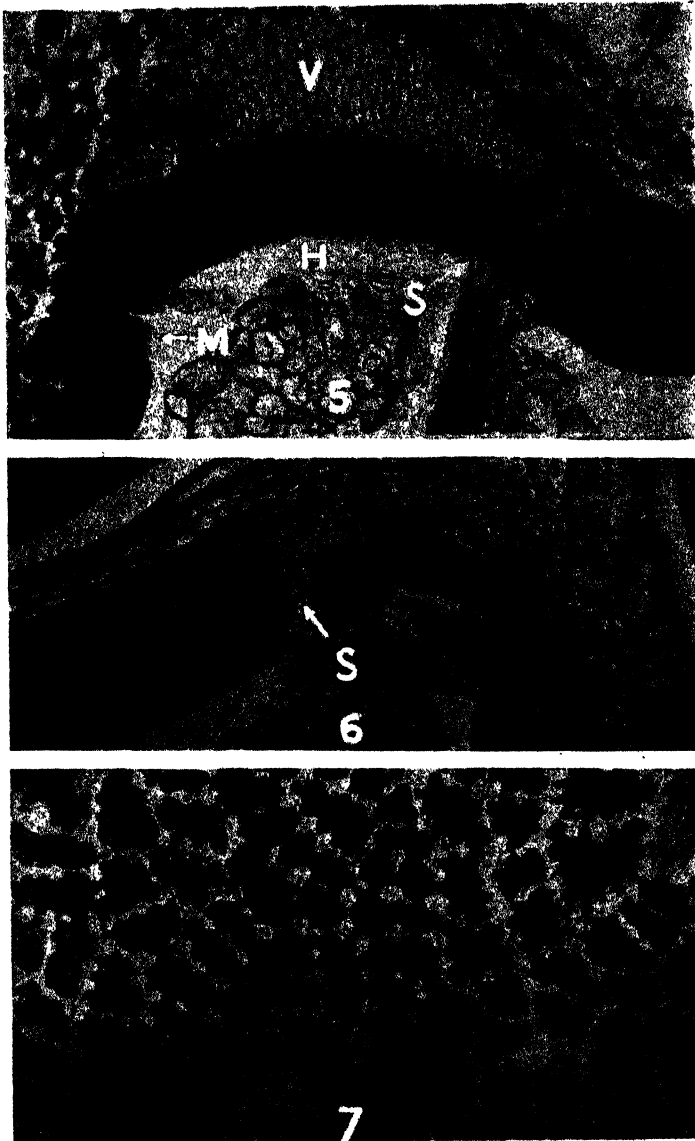
During the early development of the seed the absorption of the nucellus and inner integument is extended well back into the chalazal end of the seed. The seed coat is a product of the outer integument with the possible exception of the portion in the region of the hilum. At the time of fertilization the outer integument consists of four to six layers of cells throughout most of its extent. Its epidermal layer, consisting of almost isodiametric cells at the time of fertilization (fig. 4, a and b), becomes the malpighian layer, and the subepidermal layer becomes the osteosclerid layer of the mature seed.

In the transformation of the outer integument into a seed coat, the first perceptible modification is the radial elongation and rapid tangential division of the epidermal cells. These changes become quite noticeable as early as 72 hours after pollination, and continue until the cells are greatly increased in number and elongated to a spindle shape (fig. 4, a-m). These modifications of the epidermal as well as the modifications of other cell layers of the outer integument begin at the micropylar and chalazal ends of the seed from which they spread to the intervening portions of the respective layers.

Within the material of the rapidly thickening outer wall of the epidermal or malpighian cells, appear the cuticularized portion, domes, and light line in succession. Approximately 8 days after pollination, two parts are distinguishable—namely, the primary wall which has been called cuticle and the much thicker portion within which is the product of secondary thickening. About 5 days later the cuticularized layer and domes appear, and about 9 days later the light line is recognizable.

When fully developed and dried down to the condition attained in dry ripe seeds, the cuticularized layer, domes, and light line constitute a barrier to water, interrupted only at the micropyle and the strophiole. The micropyle, however, is very effectively closed in the sweet clover seeds as shown by the fact that in all the tests on water absorption, no seeds were observed to take water through the micropyle. In sectioning processed seeds, the pressure of the knife often caused breaks in the malpighian layer at the micropyle (fig. 5) but sections with tissues intact show the malpighian cells about the micropyle so closely united that the malpighian structures are practically continuous.

The strophiole, when examined closely in sections of processed seeds,



FIGS. 5-7. Figures 5 and 6 are radial sections through the hilum (H) portion of the seed coat, showing the micropylar (M), and strophilar (S) areas, the vascular isle region (V), and the break in the light line at the strophilar by the vascular connections. The break in the light line at the strophilar is more pronounced in the younger seed as shown in figure 6. Figure 7 shows the porous character of the endosperm cell walls.

proves to be the region of the seed coat traversed by the vascular connections between the seed and funiculus (figs. 5 and 6). The vessels of this vascular connection afford a passageway through which water can traverse the malpighian layer, when their outer ends are open.

An understanding of the operation of the strophiole in relation to water absorption must await a special study of the anatomy and physiology of this structure. It is obvious that the role of the strophiole in hardness of seeds should be recognized especially in connection with commercial scarification where the severity of the process causes much injury and considerable destruction of seeds, that could be avoided if scarification were confined to the strophiole and to the intensity barely necessary to effect softening.

ENDOSPERM

The endosperm in mature seeds completely jackets the embryo (fig. 9). It is prevailingly two-layered, but heavier over the tip of the radicle where, as a conical projection, it fills the micropylar end of the seed (fig. 8). The cell walls of the endosperm, especially those of the much thickened outer layer, are well provided with pores and traversed by protoplasmic connections, structural features which permit easy and rapid passage of water from place of entrance to all distal parts of the endosperm (fig. 7).

The contents of the endosperm cells are very largely gelatinous. When water is available they imbibe and swell energetically, a physiological feature that also facilitates a rapid distribution of water throughout the endosperm, and provides much, if not almost all, of the force that opens the seed coat.

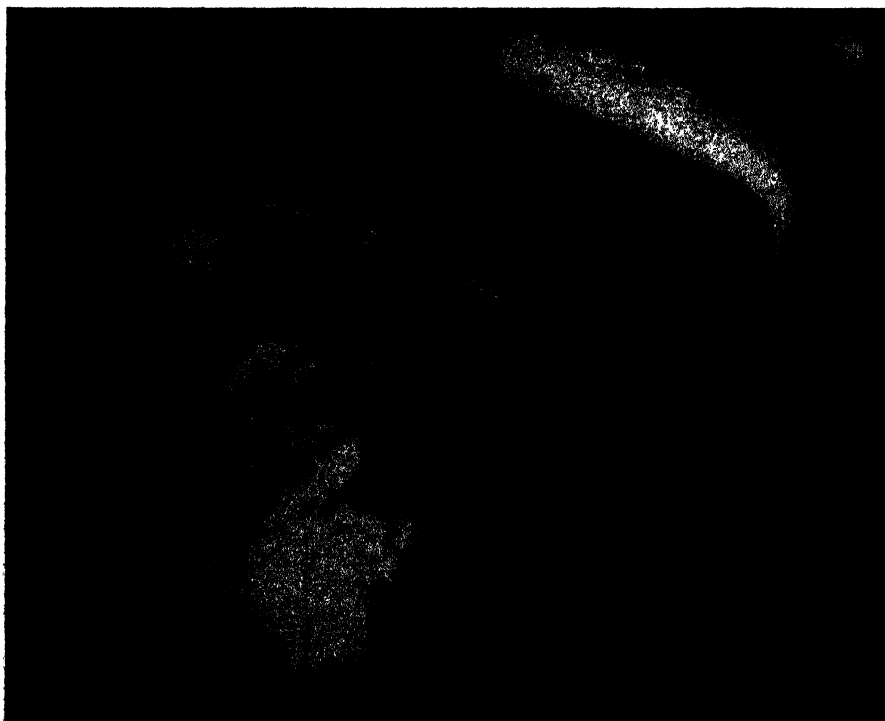
The endosperm, through its tenacious retention of water and, therefore, in functioning as a moist blanket about the embryo, no doubt has much to do with preserving the longevity of the seed during its dormancy. In the initial stage of germination, when water makes contact with the interior of the seed through the strophiole or elsewhere, its inward flow as well as its distribution to distal parts of the seed, are greatly accelerated by the imbibing force of the endosperm. Some recent investigations show that the initial stages in the germination of legume seeds is associated with chemical changes, and these may have to do chiefly with the endosperm. Certainly in sweet clover seeds the endosperm plays an important role in the life of the seed.

SUMMARY

The investigations of the germination of sweet clover seeds, including both hard and soft seeds variously processed, showed quite conclusively

FIGS. 8 and 9. Figure 8, a portion of a vertical section of a sweet clover seed, showing the malpighian (M), osteosclerid (O), and interior layers (I) of the seed coat; and the endosperm (E) and its thickened projection over the radicle (R) of the embryo.

Figure 9 shows the endosperm at the left from which the embryo at the right has been removed through the rift seen in the endosperm.



that the strophiole is the natural place of initial water absorption in the germination of sweet clover seeds. Seeds naturally soft, or softened artificially or by weathering, blackened and began swelling first at the strophiole when they were immersed in an osmic acid solution. On the other hand, hard seeds with practically no exceptions showed no blackening at the strophiole until they were softened by weathering or artificially, and then they behaved as seeds naturally soft, blackening and swelling at the strophiole in the presence of osmic acid.

Samples of hard seeds scarified by hand not too harshly were softened in proportion to the number of seeds which showed a blackening at the strophiole. The effectiveness of the scarification lay, therefore, in making the strophiole permeable to water.

Even light scarification with fine sandpaper, when directly applied to the strophiolar area, sufficed to soften the hard seeds.

In samples of seeds which had been cleaned and scarified by commercial houses, nearly all soft seeds showed blackening and water absorption at the strophiole, although many of the soft seeds also blackened and absorbed water at various other places where the seed coat had been mutilated by the processing.

The coats of soft seeds stored outside were found to deteriorate rather rapidly, many of them within 2 or 3 years, becoming permeable at one or more places other than the strophiole.

The blackening of the micropyle occurred in some seeds, both hard and soft, but no instance was observed in which water was absorbed through the micropyle.

The cuticularized layer, domes, and light line, structures formed in the secondary thickening of the outer cell walls of the malpighian layer and considered the cause of hardness, constitute almost a continuous barrier to water absorption, being interrupted only at the micropyle and strophiole.

At the micropyle, the malpighian layer closes together so tightly that the light line and associated structures are practically continuous across the orifice and make it water tight.

The so-called strophiole of sweet clover seeds was found to be the place in the hilum where the vascular connection occurs between the seed and the funiculus. At this place the light line and associated structures related to hardness are interrupted by the vascular elements, which afford a passageway for water to reach the interior of the seed.

The endosperm, consisting mainly of two gelatinous layers, completely envelops the embryo, and owing to its water retention, is well adapted to protect the embryo against excessive loss of water and temperature fluctuations. By its force of imbibition it greatly accelerates the flow of water into the seed and to all distal parts. By its pressure, exerted in swelling, it plays a major role in opening the seed coat.

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LYGUS HAHN; SIX NEW SPECIES FROM WESTERN NORTH AMERICA (HEMIPTERA, MIRIDAE)

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The growing importance of *Lygus* species as pests of alfalfa and other legume crops has prompted further studies of this genus. Six new species and one variety are described in the present paper.

Lygus desertus new species

Allied to *elisus* Van D., but distinguished by the more elongate form, more convex scutellum, shorter and finer pubescence, and by the slightly shorter rostrum. Color pale yellowish, hemelytra subtranslucent, dark specimens have outer margins of calli and two short rays behind each callus black, but apical area of corium without well-defined fuscous spots as found in *elisus*.

MALE. Length 5.8 mm., width 2.25 mm. Head: width 1.12 mm., vertex .45 mm.; yellowish, collum black, eyes brown, a small fuscous spot on gena beneath eye. Rostrum, length 2.03 mm., reaching to middle of hind coxae, yellowish, apical segment black. Antennae: segment I, length .47 mm., yellowish, fuscous beneath; II, 1.50 mm., light brown, fuscous on apex and more broadly at base; III, .78 mm., fuscous; IV, .56 mm., fuscous. Pronotum: length 1.28 mm., width at base 2.12 mm.; pale yellowish, a black spot or ray behind inner half of callus, also one behind outer half and including the margin of callus, in darker specimens the black color extends forward toward anterior angle as well as behind outer margin of callus. Scutellum rather strongly convex, rather coarsely transversely rugulose punctate, pale to yellow, middle of base invaded by black color of mesonotum.

Hemelytra elongate, costal margins subparallel, pallid, subtranslucent, a bit of fuscous on middle of clavus but separated by claval vein; in darkest specimens a bit of fuscous bordering radial vein on apical area of corium but never with a well-defined large fuscous patch as in *elisus* Van D. Cuneus pallid, narrow apex fuscous. Membrane clear, veins white, a strong callosity bordering inner apical angle of cubitus. Dorsum bearing very fine short pubescence, much less conspicuous than in *elisus*. Venter yellow to greenish, without dark marks. Pleura yellowish, sternum fuscous. Legs pale to yellowish, femora with two subapical fuscous rings, hind pair sometimes with fuscous shading on middle; hind tibiae with mark on knee and oblique band just beneath, fuscous, spines black; tarsi yellowish brown, apices and claws blackish. Genital claspers rather similar to those of *elisus* and while small differences are apparent, we do not find these structures very practical in sorting the species.

FEMALE. Length 6.00 mm., width 2.7 mm.; more robust than the male but very similar in color and pubescence. Head: width 1.21 mm., vertex .52 mm. Rostrum, length 2.07 mm., just reaching to middle of hind coxae. Antennae: segment I, length .47 mm.; II, 1.47 mm.; III, .78 mm.; IV, .52 mm. Pronotum: length 1.38 mm., width at base 2.38 mm. The black rays on calli usually shorter than in male; pale specimens may have only one black spot behind each callus, and in this case suggestive of *elisus* but the corium will be clear of fuscous.

Mr. L. L. Stitt reports that in the field living specimens may be distinguished from *elisus* by their peculiar shining appearance. We believe this aspect is due to the very fine short pubescence and the pallid translucent color.

HOLOTYPE: ♂ April 10, 1942, Ajo, Arizona (Lloyd L. Stitt); author's collection. **ALLOTYPE:** same data as the type. **PARATYPES:** 1 ♂ 7 ♀, taken with the types on *Sphaeralcea* sp. ARIZONA—Arcadia: ♀ Feb. 11, 1937 (L. L. Stitt). Aztec: ♂ ♀ March 19, 1941 (L. L. Stitt). Cave Creek: 4 ♂ 2 ♀ April 19, 1936 (L. L. Stitt); reared from *Astragalus diphysus*. Datan: ♂ ♀ April 14, 1939 (L. L. Stitt), on *Abronia* sp. Dome: 2 ♂ 1 ♀ April 15, 1937 (L. L. Stitt), on *Actinea odorata*. Fresno Village: ♂ April 10, 1942 (L. L. Stitt). Laveen: ♀ April 6, 1937 (L. L. Stitt). Liberty: ♀ April 26, 1937 (L. L. Stitt). Long Valley: ♂ ♀ August 22, 1940 (L. L. Stitt). Phoenix: 5 ♀ March 17, 1939; ♀ March 9, 2 ♂ 1 ♀ May 1, 1942 (L. L. Stitt). Prescott: 2 ♂ 1 ♀ May 28, 1939 (L. L. Stitt). Sacaton: ♂ May 6, 1931 (E. D. Ball). Tempe: ♂ Feb. 16, 1939, ♂ ♀ March 5, 1942 (L. L. Stitt). CALIFORNIA—♂ Aug. 24, 1935, 20 miles northwest of Alturas (Joe Schuh). COLORADO—Gunnison: ♂ ♀ August 17, 1925 (H. H. Knight) on *Chrysothamnus* sp. IDAHO—Bruneau: 2 ♂ 8 ♀ June 23, 1938 (H. M. Harris). Eureka: 2 ♂ June 23, 1938 (H. M. Harris). Hailey: ♂ ♀ June 25, 1938 (H. M. Harris). Hansen: ♂ August 3, 1938 (H. M. Harris). Henry's Lake: ♂ July 9, ♀ July 12, alt. 5,000 ft. (R. E. Miller). Hollister: ♂ 3 ♀ August 4, 1938 (H. M. Harris). Lewiston: ♂ April 27, 1936 (R. E. Miller). Moscow: ♂ April 14, 2 ♂ April 18, 1933 (T. A. Brindley). Mountain Home: 20 ♂ ♀ June 23, 1938 (H. M. Harris), on *Chrysothamnus*. Regina: 4 ♂ 7 ♀ June 23, 1938 (H. M. Harris). St. Anthony: 9 ♂ ♀ May 6, 1933 (W. E. Shull). NEVADA—Glendale: 2 ♂ 1 ♀ Sept. 18, 1929 (David E. Fox), on *Chrysothamnus paniculata*. OREGON—Fort Klamath: ♂ Aug. 11, ♂ Aug. 31, 1930, alt. 4,200 ft. (H. A. Scullen). Klamath Falls: ♂ ♀ July 24, 1930 (59 miles east) (H. A. Scullen). Sisters: 8 ♂ ♀ Aug. 7, 1935, alt. 3,180 ft. (H. A. Scullen), on *Chrysothamnus*. UTAH—Emery County: ♂ Aug. 10, 2 ♂ Aug. 14, ♂ Aug. 20, 1921 (Grace O. Wiley). Leeds: 2 ♂ Oct. 12, 1932 (E. W. Davis). Millard County: 2 ♂ 1 ♀ May 29, 1940, White Valley (V. M. Tanner). St. George: 2 ♀ June 10, 1928 (E. W. Davis). Vernal: ♂ July 14, 1927 (G. F. Knowlton). WYOMING—Farson: ♂ ♀, Big Sandy Creek (V. M. Tanner).

Lygus nigritus new species

Allied to *columbiensis* Kngt., but form more robust and rostrum slightly longer; the black color is suggestive of *oblineatus* var. *strigulatus*

Walker, but claw of right clasper is different and the frons does not show the double vittate mark.

MALE. Length 5.5 mm., width 2.5 mm. Head: width 1.10 mm., vertex .43 mm.; chiefly black, frons and vertex yellowish, bordering inner margins of eyes and an inverted "V" on middle of frons, blackish, eyes dark brown. Rostrum, length 2.38 mm., reaching upon fourth ventral segment, brownish to black. Antennae: segment I, length .56 mm., black; II, 1.73 mm., black, paler specimens more brownish; III, .82 mm., black; IV, .57 mm., black; clothed with fine, suberect, yellowish pubescence. Pronotum: length 1.21 mm., width at base 2.03 mm.; disk moderately convex, rather coarsely punctate, black, shining, paler specimens show rays behind calli; basal edge, median line, inner half of calli, before calli, collar, and margins of propleura, pale to yellowish brown. Scutellum black, apex and vittate mark each side at base, pale, in lighter specimens the pale marks may join to form a "Y"; convex, coarsely rugose punctate on middle and sides. Hemelytra black, subtranslucent on middle and on apex of clavus; base and more or less on middle of apical half of corium, apex of clavus, and cuneus except margins, pale yellowish and subtranslucent. Membrane pale, middle of apical half and apices of areoles shaded with fuscous. Dorsum clothed with short recumbent, yellowish pubescence, these hairs arising from distinct punctures.

Venter black, spots inclosing spiracles, and a broad lateral stripe on each side, yellowish. Legs chiefly black, femora with a pair of subapical annuli, more obscure on hind pair; tibiae pale to yellowish, blackish on basal half but with an oblique white mark near base. Genital claspers rather similar to *columbiensis* Kngt., but claw of right clasper extending distally, then sloping downward at an oblique angle.

HOLOTYPE: ♂ July 6, 1937, Dayton, Washington (R. E. Miller); author's collection. **Paratypes:** 3 ♂ Sept. 7, 1942, Salinas, California (W. H. Lange), on *Parthenium argentatum* Gray.

Lygus varius new species

Allied to *shulli* Kngt., but second antennal segment shorter, rostrum slightly longer and dorsum more strongly pubescent; color yellowish brown, strongly marked with fuscous and black.

MALE. Length 5.6 mm., width 2.8 mm. Head: width 1.17 mm., vertex .51 mm.; yellowish and marked with black; bordering inner margin of eye and projecting into vertex above, and an inverted V-shaped mark on middle of frons, black; upper half of juga, upper and lower margins of lora, gula, and apical half of tylus, black. Rostrum, length 2.6 mm., extending upon fourth ventral segment, brownish, apex black. Antennae: segment I, length .52 mm., yellowish brown, fuscous on base; II, 1.56 mm., brownish, apical half and band at base black; III, .78 mm., blackish; IV, .60 mm., blackish; clothed with fine pale pubescence. Pronotum: length 1.25 mm., width at base 2.16 mm.; disk moderately convex, lateral margins straight, anterior angles prominent, disk rather evenly but strongly punctate, yellowish brown, calli except inner half, two vittae behind each callus and one behind anterior angle and bordering lateral margin, black,

inner pair of vittae usually short; collar and propleura pale yellowish, two flaring rays across top of coxal cleft blackish. Scutellum moderately convex, rather strongly transversely rugulose; yellowish, a triangular black mark each side of median line, within each triangle a pale vitta arising at base; mesoscutum black.

Hemelytra with lateral margins arcuate, closely punctate and pubescent; yellowish translucent, clavus and corium with streaks and spots of fuscous; cuneus yellowish translucent, apex, outer edge and basal angle blackish. Membrane fuscous, veins and basal area of areoles paler. Venter blackish, genital segment more brownish, a series of yellowish spots on lateral median line, spiracles surrounded by small pale spots. Right genital clasper with a broadly curved claw on apex. Legs yellowish to brownish and obscured by fuscous; tibiae yellowish, blackish at base but broken by an oblique pale annulus; tarsi brownish, apical segment blackish.

FEMALE. Length 5.3 mm., width 2.7 mm. Head: width 1.12 mm., vertex .47 mm. Antennae: segment I, length .52 mm.; II, 1.47 mm.; III, .78 mm.; IV, .60 mm. Pronotum: length 1.17 mm., width at base 2.17 mm. Very similar to the male in coloration but with black markings much reduced; the ground color more reddish brown than yellowish, the black rays on pronotum nearly obsolete but markings on scutellum typical.

HOLOTYPE: ♂ August 22, 1925, Pingree Park, Colorado (H. H. Knight); author's collection. **ALLOTYPE:** ♀ August 14, 1931, Mt. Rainier, Washington (H. H. Knight). **PARATYPES:** ♂, taken with holotype; ♀, taken with allotype. **ALBERTA**—♀ Aug. 10, 4 ♂ Aug. 12, 1929, Banff (Owen Bryant). ♂ Aug. 6, 1921, Nordegg (J. McDunnough). **WYOMING**—♂ Aug. 15, 1938, Centennial (E. Hixson). ♂ Aug. 8, 1935, Green River Lake (H. Ruckes).

***Lygus fultoni* new species**

Allied to *robustus* Uhler, but differs in the larger size, paler color, shorter second antennal segment, lateral margins of pronotum more acutely angled, frons and scutellum less convex.

MALE. Length 6.6 mm., width 2.9 mm. Head: width 1.21 mm., vertex .48 mm., yellowish, median line of frons and sutures about juga, reddish. Rostrum, length 2.33 mm., just reaching middle of hind coxae, yellowish brown, apex black. Antennae: segment I, length .60 mm., yellowish brown, blackish beneath; II, 1.86 mm., black, brownish above on basal half, yellowish pubescent; III, .91 mm., black; IV, .69 mm., black. Pronotum: length 1.43 mm., width at base 2.51 mm.; evenly convex, distinctly and evenly punctate, lateral margins nearly straight, angle with propleura distinct; pallid to yellowish, calli brownish, a rounded spot behind inner margin, a larger spot on outer margin and joining with spot near anterior angles of disk, black; propleura pale to yellowish, a black ray behind top of coxal cleft. Scutellum moderately convex, transversely rugose, pale yellowish, median line with broad black wedge extending from base to near middle of disk, bifid on apex, mesoscutum black.

Hemelytra somewhat elongate, costal margin slightly arcuate, pallid

to yellowish translucent, clothed with very fine short pubescence; apical area of corium with two fuscous patches, also two spots on apical margin bordering membrane; cuneus evenly translucent, apex more yellowish but not fuscous. Membrane pale, slightly infuscated on apical half and within apex of areoles, veins yellowish. Venter and propleura yellowish, tinged with reddish, mesosternum fuscous on middle. Legs yellowish, with indistinct subapical annuli; mark on base of tibiae and tips of tarsi fuscous. Right genital clasper with broadly curved terminal claw.

FEMALE. Length 5.8 mm., width 2.9 mm. Head: width 1.30 mm., vertex .52 mm. Antennae: segment I, length .60 mm.; II, 1.68 mm., black, more brownish above; III, .91 mm.; IV, .69 mm. Pronotum: length 1.40 mm., width at base 2.42 mm. More robust than the male but very similar in color and pubescence.

HOLOTYPE: ♂ August 25, 1926, North Park, Colorado (B. B. Fulton); author's collection. **ALLOTYPE:** same data as the type. **PARATYPES:** 2 ♂ taken with the types. **IDAHO**—♀ May 19, 1936, Lewiston, alt. 550 ft. (T. A. Brindley).

***Lygus brindleyi* new species**

Allied to *hesperus* Kngt., but differs in being nearly glabrous, scutellum more convex and with coarse punctures, second antennal segment shorter and somewhat thicker; color pallid to yellowish, second antennal segment, mesoscutum, and outer half of calli black.

MALE. Length 5.5 mm., width 2.7 mm. Head: width 1.14 mm., vertex .45 mm.; uniformly pale yellowish without markings, collum black. Rostrum, length 2.47 mm., extending to fourth ventral segment, yellowish, apical half brownish, apex black. Antennae: segment I, length .56 mm., brownish, black beneath; II, 1.83 mm., black; III, .91 mm., black; IV, .60 mm., black. Pronotum: length 1.34 mm., width at base 2.25 mm.; disk evenly convex, coarsely, and rather closely punctate; lateral margins of disk forming distinct angle with propleura; uniformly pale yellowish, calli with small spot just behind inner half, outer third of callus and extending forward to collar but not including anterior angle, shining black, also a black spot just behind top of coxal cleft. Scutellum distinctly convex, coarsely punctate, rugose, a bifid black triangle on middle of base; mesoscutum black.

Hemelytra with costal margins slightly arcuate, shallowly punctate, a very fine, short, pale pubescent hair arising from each puncture, but the dorsal aspect giving a glabrous impression; uniformly pale yellowish, a translucent line along radial vein and a small fuscous mark bordering apical end of this; cuneus yellowish, opaque except for a translucent line bordering lateral edge, and this appears to be an extension of costal vein. Membrane pale fuscous, veins yellowish opaque, white on apex, a small callus spot bordering apex of larger areole. Venter uniformly pale yellowish, somewhat brownish on sides; pleura and sternum uniformly yellowish. Legs yellowish, a pair of subapical fuscous bands on femora, stronger on hind pair; tibiae yellowish, spines black, a black mark at base;

tarsi fuscous, apex and claws black. Right genital clasper with terminal claw broadly curved.

FEMALE. Length 5.4 mm., width 2.6 mm. Head: width 1.21 mm., vertex .49 mm. Antennae: segment I, length .52 mm.; II, 1.64 mm.; III, .84 mm.; IV, .60 mm. Pronotum: length 1.30 mm., width at base 2.2 mm. Very similar to the male in form and coloration, although the black marks about the calli are obsolete.

HOLOTYPE: ♂ August 15, 1936, alt. 2,560 ft., Moscow, Idaho (T. A. Brindley); author's collection. **ALLOTYPE:** same data as the type. **PARATYPES:** ♂ ♀ taken with the types. ♀ July 12, 1936, alt. 7,000 ft., Henry's Lake, Idaho (R. E. Miller). **WASHINGTON—**♂ Sept. 3, 1931, Tampico (A. R. Rolfs). ♀ Sept. 15, 1938, Wenatchee (John Standish).

***Lygus oregonae* new species**

Allied to *bradleyi* Kngt., about the same size and color, but distinguished by the longer rostrum which extends beyond posterior coxae.

MALE: Length 5.5 mm., width 2.7 mm. Head: width 1.08 mm., vertex .476 mm.; yellowish, an inverted "V" on frons and connected with a small triangle on middle of vertex, a vertical mark above base of antenna, mark across middle of juga, upper margin of lora, upper margin of buccula, gula, apex, and mark on middle of tylus, black; frons striate, pale pubescent. Rostrum, length 2.65 mm., extending upon fourth ventral segment, yellowish brown, apical half blackish. Antennae: segment I, length .39 mm., brown, blackish beneath; II, 1.21 mm., yellowish, base and apical one-fourth blackish, finely pale pubescent; III, .52 mm., blackish; IV, .39 mm., blackish. Pronotum: length 1.17 mm., width at base 2.25 mm.; disk more convex than in *bradleyi*; distinctly and rather closely punctate, with short pale pubescence; yellowish brown, nearly orange on anterior margin and collar, spots on inner and outer margins of calli, a pair of rays behind each callus, bordering lateral margin and a distinct spot on basal angle of disk, black; propleura pale to yellowish, a blackish triangle on central area; mesoscutum covered, scutellum black, having a Y-shaped pale area. Hemelytra with costal margins slightly arcuate on distal half, closely punctate, pale pubescent; pale translucent, mottled with fuscous patches much as in *bradleyi*, but a stronger mark on apex of corium; cuneus pale translucent, narrow edge and apex darkened. Membrane pale, apical half and apex of areoles infuscated. Venter yellowish, ventral half dark fuscous; right genital clasper nearly quadrate, apical claw strongly decurved. Pleura yellowish, sternum black. Legs pale yellowish and marked with black, femora broadly on middle and two subapical annuli blackish; tibiae pallid, spines dark brown, spot on base and oblique annulus just below, blackish; tarsi brownish.

FEMALE. Length 5.3 mm., width 2.34 mm. Head: width 1.05 mm., vertex .476 mm. Antennae: segment I, length .39 mm.; II, 1.17 mm.; III, .56 mm.; IV, .47 mm. Pronotum: length 1.10 mm., width at base 2.12 mm. Very similar to the male in color and pubescence.

HOLOTYPE: ♂ August 18, 1929, Waldport, Oregon (J. E. Davis); author's collection. **ALLOTYPE:** same data as the type.

***Lygus ceanothi rufus* new variety**

Apparently not differing structurally from *ceanothi* Knegt., but the distinctly reddish color gives an aspect quite different from the typical species.

MALE. Length 6.3 mm., width 2.9 mm. Head: width 1.17 mm., vertex .43 mm. Rostrum, length 2.94 mm., reaching to base of fifth ventral segment. Antennae: segment I, length .65 mm.; II, 2.1 mm.; III, .95 mm.; IV, .73 mm.; reddish, last two segments fuscous. Pronotum: length 1.3 mm., width at base 2.33 mm.; reddish brown, a small spot behind each callus, and a large rounded spot on basal angles of disk, black. Mesoscutum black, scutellum red, a Y-shaped yellowish mark evident. Hemelytra subtranslucent, reddish, deeper red on embolium and margins of cuneus, veins red. Legs brown to reddish.

FEMALE. Length 6 mm., width 3 mm. Head: width 1.21 mm., vertex .47 mm. Antennae: segment I, length .60 mm.; II, 1.9 mm.; III, .95 mm.; IV, .65 mm. Pronotum: length 1.4 mm., width at base 2.4 mm. Very similar to the male in coloration.

HOLOTYPE: ♂ June 21, 1932, Tieton Canyon, Washington (A. R. Rolfs); author's collection. **ALLOTYPE:** same data as the type. **PARATYPES:** 7 ♂ ♀, taken with the types on *Ceanothis sanguineus*. 12 ♂ ♀ July 4, 1932, Signal Park, Wash. (A. R. Rolfs). ♂ July 15, 1932, Mt. Adams, Wash. (A. R. Rolfs).

* * *

***Lygus ceanothi deleticus* Knight.**

Iowa State Col. Jour. Sci., xv, 1941, p. 270.

In the original publication a printer's error occurs as this varietal name was spelled "delecticus"; obviously a spelling never intended by the author.

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